

AD \_\_\_\_\_

Award Number:

W81XWH-07-1-0129

TITLE:

**PHARMACOLOGICAL PREVENTION AND REVERSION OF ERECTILE  
DYSFUNCTION AFTER RADICAL PROSTATECTOMY, BY MODULATION OF  
NITRIC OXIDE/cGMP PATHWAYS**

PRINCIPAL INVESTIGATOR:

Nestor F. Gonzalez-Cadavid, PhD

CONTRACTING ORGANIZATION:

Charles Drew University  
Los Angeles, CA 90059

REPORT DATE:

March 2010

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
1. REPORT DATE (DD-MM-YYYY) 01-03-2010		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 1 MAR 2009 - 28 FEB 2010	
4. TITLE AND SUBTITLE  PHARMACOLOGICAL PREVENTION AND REVERSION OF ERECTILE DYSFUNCTION AFTER RADICAL PROSTATECTOMY, BY MODULATION OF NITRIC OXIDE/cGMP PATHWAYS			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER W81XWH-07-1-0129		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Nestor F. Gonzalez-Cadavid			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Charles Drew University (CDU)  Los Angeles, CA 90059			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Fort Detrick, Maryland 21702-5012			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT During years 1 and 2 we have shown that long-term sustained administration of high doses of PDE5 inhibitors (tadalafil and sildenafil, previously vardenafil), prevented in a rat model of cavernosal nerve damage post-radical prostatectomy the erectile dysfunction (corporal veno-occlusive dysfunction or CVOD) and the underlying penile corporal histopathology resulting from this surgery for prostate cancer in men. Now, we have shown that much lower doses of sildenafil, combined or not with a nitric oxide donor, molsidomine, also correct the CVOD, although so far the stimulation of smooth muscle repair and decrease of fibrosis in the corpora cavernosa do not seem to occur (ongoing assays). We have also studied in this model the effect of implanting muscle derived stem cells (MDSC) into the corpora in the presence or absence of low dose sildenafil (ongoing). During the no-cost extension we aim to complete these studies and determine whether sildenafil stimulates nerve terminal regeneration in the corpora, and, if possible, examine a complementary hypothesis to explain the correction of CVOD by low doses of sildenafil based on secondary tissue targets. Our results pioneered and provide a scientific justification for the emerging shift from the current "on demand/sporadic" therapy of post-radical prostatectomy patients with PDE5 inhibitors, towards their long-term daily administration in the novel "penile preservation post-radical prostatectomy" clinical approach.					
15. SUBJECT TERMS Radical prostatectomy, quality of life, erectile dysfunction, PDE5 inhibitors					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT  UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)

## Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	2
Reportable Outcomes.....	7
Conclusion.....	8
References.....	9.
Appendices.....	10

## **Introduction**

Our original hypothesis was that erectile dysfunction (**ED**) subsequent to radical prostatectomy for prostate cancer can be prevented and even reversed by long-term sustained treatment with PDE5 inhibitors, alone or in combination with nitric oxide (**NO**) generators, that combat oxidative stress and fibrosis in the penile corpora cavernosa smooth muscle and stimulate cavernosal nerve regeneration, due to the vasculo- and neuroprotective effects of NO and/or its reaction product, cGMP. We aimed to study in a bilateral cavernosal nerve resection (**BCNR**) rat model whether:

- 1) There is a progressive increase in collagen synthesis and SMC apoptosis in the corpora cavernosa trabecular tissue compounded by a decrease in collagen breakdown, due to production of pro-fibrotic factors such as TGF $\beta$ 1, that leads to a progressive corporal veno-occlusive dysfunction (**CVOD**), the primary form of vasculogenic ED.
- 2) The induction of inducible nitric oxide synthase (**iNOS**) which is the tissue's endogenous mechanism of defense against fibrosis by its inhibition of collagen synthesis through NO reduction of reactive oxygen species (**ROS**) produced during oxidative stress, can be mimicked by long-term oral treatment with NO generators and/or PDE5 inhibitors, and thus prevent or reverse CVOD, due to the vasculoprotective effects of these agents that increase tissue compliance.
- 3) These agents lead also to partial penile nerve regeneration, due to their neuroprotective effects.

Our specific aims were:

**Aim 1. To assess the most effective adjuvant therapy to radical prostatectomy to prevent smooth muscle (SM) fibrosis and CVOD, by the modulation of the NO/cGMP/ROS balance in the penis subsequent to cavernosal nerve damage in the rat model.**

**Experiment 1. Time-course of BCNR induction of CVOD and the underlying penile SM fibrosis. Experiment 2. Effects of sustained oral administration of PDE5 inhibitors or NO generators on CVOD and the underlying corporal fibrosis, at a selected time after BCNR**

Rats will be subjected to BCNR or sham-operated, injected in the corpora with a collagen I promoter- $\beta$  galactosidase construct (**Col I-Pr-Bgal**) and bromodeoxyuridine (**BrdUr**), 7 days and 20 hs before sacrifice, respectively, and sacrificed at 10, 14, 30, 60, 90 days, or only at 90 days after continuous oral treatment with: 1) PDE5 inhibitor (vardeafil), 2) NO donor (molsidomine), or 3) vardeafil/molsidomine combination. **Total: 136 rats. Outcomes:** a) CVOD, by dynamic infusion cavernosometry (**DIC**); b) fibrosis in corpora and SM by quantitative image analysis (**QIA**) in tissue sections: SM/collagen (Masson), collagen III/I (Sirius red), SMC content (ASMA); c) fibrosis in tissue homogenates: collagen (hydroxyproline), ASMA (western blot).

**Aim 2. To optimize the selected treatment to prevent or reverse CVOD after cavernosal nerve damage, and to determine its effect on collagen and SMC turnover, the oxidative stress/nitrosative reaction, and nitrergic nerve regeneration.**

**Experiment 3. Comparison of treatment modalities involving PDE5 inhibitors. Experiment 4. Time course of BCNR effects on collagen and SMC turnover rates, oxidative stress, and nitrosative reaction, and modulation of these processes by selected PDE5 inhibitor. Experiment 5. Effects of selected treatment on nerve regeneration**

New rats operated on as in Aim 1 will be also injected 20 h hours prior to sacrifice with BrdUr, and submitted for 90 days to long-term oral treatments with: 1) continuous vardeafil at half the previous dose, 2) as in #1, but with molsidomine (an NO donor); 3) once a day vardeafil; 4) once a day vardeafil plus molsidomine; 5) once a day pentoxifylline, as PDE4 inhibitor; 6) continuous L-arginine, as NOS substrate; 7) selected treatment, but at 45 days post-

BCNR for 45 days. In addition, the following 90-day treatment groups will receive also 2 days before sacrifice fluorogold 4% for transneuronal retrograde tracing: 8) BCNR with selected treatment; 9) BCNR untreated; 10) sham untreated. **Total: 85 rats.** Outcomes: Groups 1-8 will be assayed for: a) as in Aim 1; b) collagen turnover by luminometry & matrix metalloproteinase (**MMP**) activity; c) SMC turnover by QIA for BrdUr and apoptosis; d) Oxidative/nitrosative balance by QIA and western blot for xanthine oxidoreductase (**XOR**), iNOS, and peroxynitrite. Groups 8-10 will be assayed for: e) regeneration of nitrergic nerves by immunohistochemistry in pelvic ganglion (**IHC**) for PRV/ PnNOS; f) neurofilaments by synaptophysin by IHC/QIA; g) neuron body regeneration by BrdUr-IHC.

Some modifications throughout the project included replacing vardenafil by sildenafil, bromo-deoxyuridine by proliferating cell nuclear antigen (**PCNA**) and other minor changes. During Year 3 we complied with Experiments 3 and part of 4, since the other part of Experiment 4 was the subject of Abstract A-2, that is included in this report as paper P-8, and is therefore completed. Our new experimental work describes below different treatment modalities in the BCNR rat model for erectile dysfunction subsequent to radical prostatectomy for prostate cancer, based on the long term sustained administration of PDE5 inhibitors. Our previous results used doses of sildenafil in the rat model that when translated to men would be equivalent to, or lower than, the ones used for pulmonary hypertension.

This was aimed to check whether reducing the PDE5 inhibitor dose, and combining it with a nitric oxide donor (molsidomine), still achieves a similar amelioration of CVOD and of the underlying histopathology as compared to our previous publications with high dose during Years 1 and 2. This would help to develop a pharmacological therapy aiming to cure this type of ED with the minimum of side effects, and not just to palliate it, in the new approaches for "penile preservation post-radical prostatectomy" that are now being introduced in the clinic. Therefore, our results have some translational impact in a topic of considerable public health significance.

In addition, we have initiated studies aimed to determine whether our previous demonstration that muscle derived stem cells (**MDSC**) can correct ED associated with aging (Nolazco G, Kovancz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int, 101(9):1156-64) can be successfully replicated in the BCNR model, and whether combination with a low dose PDE5 inhibitor stimulates the MDSC conversion into corporal smooth muscle cells.

Finally, we have complemented these studies with the parallel ones in another model of erectile dysfunction, the diabetic mouse model lacking inducible nitric oxide synthase (iNOS), where we tested the effects of molsidomine on the underlying histopathology, and we have discussed them in relation to another fibrotic condition of the penis where long-term sustained administration of PDE5 inhibitors may have therapeutic value.

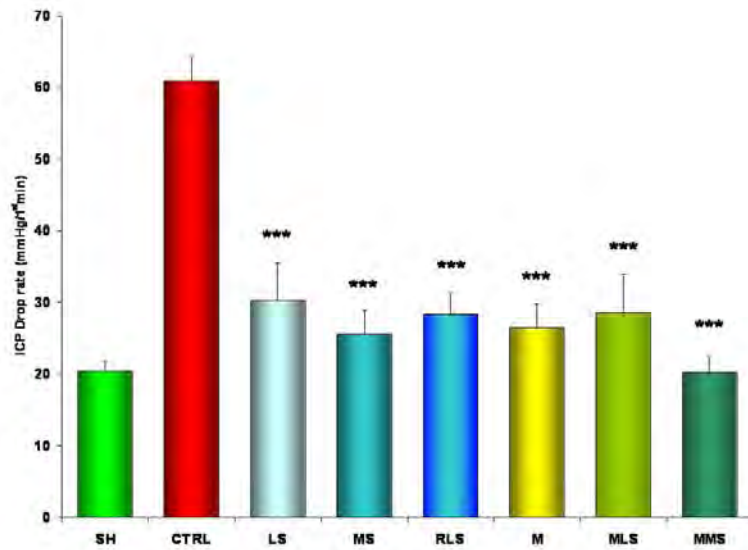
A no-cost extension was issued by DOD to complete the ongoing assays for the experiments mentioned above, and particularly for addressing experiment 5 on the specimens already collected. This no-cost extension may even allow to do some preliminary assays to examine a complementary hypothesis for the restoration of erectile function by the regimen we have investigated.

### **Description of research accomplishments**

#### **A. For Aim 2 Experiment 3. Comparison of treatment modalities involving PDE5 inhibitors.**

The aim was to optimize the regimen by reducing doses and by combining with long-action NO donors that would stimulate cGMP levels. Rats were subjected to BCNR and divided in the following long-term sustained treatment groups. These are the last animal experiments for this

grant and they have just finalized in terms of the functional determinations, but some of the data is still being analyzed, and in addition several assays are still pending and will be completed before the end of the no-cost extension.



**Fig. 1. Effect of long-term sustained treatments with sildenafil, combined or not with molsidomine, on CVOD induced by BCNR**

tablet intake by men.

5) BCNR with LS sildenafil, as retrolingual single daily instillation of 0.2 ml of a special formulation at 5 mg/ml, 1 mg/day, or approximately 2.5 mg/kg/day, or "RLS", about 25 mg equivalent tablet intake by men

6) As in #3, plus molsidomine 10 mg/kg/day (IP), or "MMS"

7) As in #4, plus molsidomine 10 mg/kg/day (IP), or "MLS"

8) BCNR with molsidomine alone at 10 mg/kg/day, or "M"

#### Series B

9) BCNR with MDSC injected into the corpora ( $10^6$  cells), or "MDSC"

10) as in 9), with low sildenafil, or "MDSC-LS"

**Figure 1** shows that all sildenafil treatments at lower doses or in discontinuous administration, or molsidomine alone or in combination with sildenafil, normalized the impaired erectile response of the BCNR rats measured by the drop rate during dynamic infusion cavernosometry. The higher the drop rate in comparison with sham-operated rats the more intense CVOD is. This is a positive

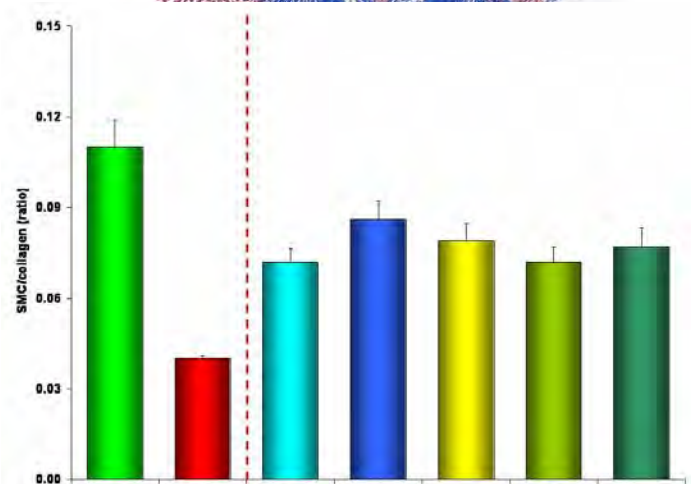
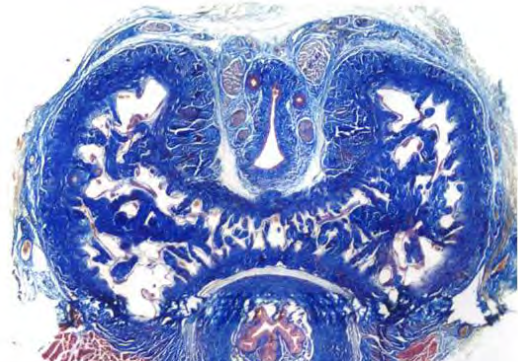
#### Series "A"

1) Sham, or "SH"

2) Untreated BCNR, or "control"

3) BCNR with continuous oral sildenafil, in the drinking water at a medium dose (1/2 the previous dose, approximately 10 mg/kg/day), or "MS", about 100 mg equivalent tablet intake by men.

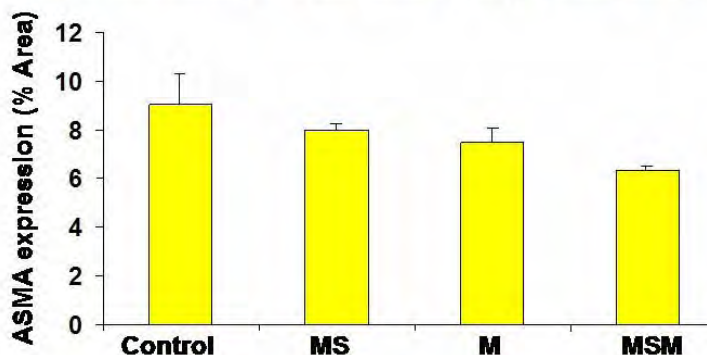
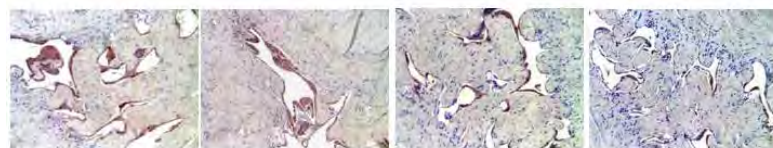
4) BCNR with continuous oral sildenafil, in the drinking water at a low dose (1/8 the previous dose, approximately 2.5 mg/kg/day), or "LS", about 25 mg equivalent



**Fig. 2. Effect of long-term sustained treatments with sildenafil, combined or not with molsidomine, on the reduction of the corporal smooth muscle collagen ratio induced by BCNR**

result that is promising because it suggests that even at these low dosages, long-term sustained sildenafil is able to improve erectile function by possibly acting on the underlying histopathology.

**Figure 2** suggests that this is the case, since the corporal SMC/collagen is moderately increased by the different treatments. However, three factors need to be addressed before the



**Fig. 3.** Effect of long-term sustained treatments with sildenafil, combined or not with molsidomine, on the reduction of the corporal smooth muscle induced by BCNR, estimated by immunohistochemistry

comparison is completed) no significant difference against the control, and even a slight trend to a reduction. The image analysis for the other groups is ongoing. A similar situation occurred with the western blot for ASMA, where so far, and unexpectedly too, no significant differences have been found (**Figure 4**). These determinations are being repeated with new specimens because of inconsistencies in the GAPDH correction.

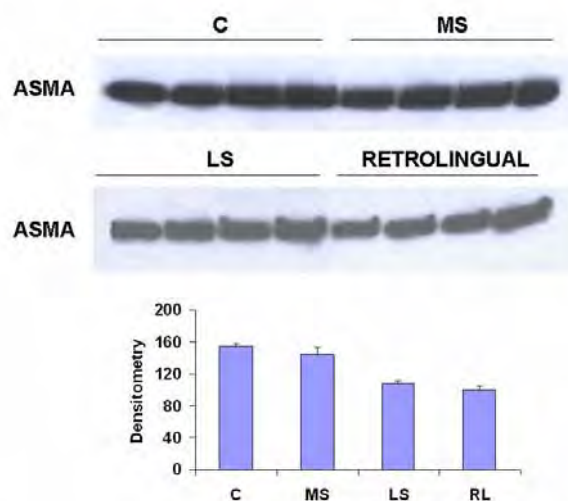
As to the experiment involving MDSC alone or supplemented with low sildenafil, the cavernosometry results look promising, but since they are ongoing we will present them on the final report.

In addition, we conducted related studies to support the potential antifibrotic effects of long-term sustained administration of PDE5 inhibitors, focusing on two other fibrotic conditions of the penis that involve similar fibrotic and antifibrotic pathways. One study is the localized fibrotic plaque of the tunica albuginea of the penis reported in the review paper P-7. The other is the investigation of the effects of molsidomine on penile corporal fibrosis induced by diabetes, utilizing a transgenic mouse where iNOS is genetically blocked (P-8). Both papers support our approach in the BCNR rat model with both types of agents.

statistical analysis and reaching any conclusion: a) the control group here is taken from ref. P1, since the assay of the true control group in this experiment is still ongoing; b) the previously reported increase with higher sildenafil dosages (ref. P2) was much more considerable; c) the immuno-histochemistry (Fig. 3) and western blot analysis (Fig. 4) of alpha smooth muscle actin (**ASMA**) expression, an indicator of SMC content do not show any significant increase by sildenafil.

In fact **Figure 3** shows for the

groups with medium sildenafil dose and with molsidomine, or with molsidomine alone (the ones were the quantitative



**Fig. 4.** Effect of long-term sustained treatments with sildenafil, combined or not with molsidomine, on the reduction of the corporal smooth muscle induced by BCNR, estimated by western blot

**Summary of paper P-7. Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy.**

Peyronie's disease (PD) is a prevalent localized fibrotic condition of the penile tunica albuginea, associated with risk factors for corpora cavernosa fibrosis (aging and diabetes), and with another localized fibrotic process (Dupuytren's disease). Most of the current pharmacological treatments for PD are not based on antifibrotic approaches substantiated by prior evidence in animal models or clinical efficacy in any other fibrotic condition, and this may explain why they are not generally successful. Evidence gathered on human specimens and animal models of PD have elucidated aspects of its etiology and histopathology, showing that overexpression of TGF $\beta$ 1, plasminogen activator inhibitor 1, reactive oxygen species, and other profibrotic factors, assumed to be induced in most cases by trauma to the tunica albuginea, leads to myofibroblast accumulation and excessive deposition of collagen. In parallel, a steady overexpression of inducible nitric oxide synthase (**iNOS**), and hence of nitric oxide and cGMP, appears to act as an antifibrotic mechanism. This process has also been reported in corporal and cardiovascular fibrosis, and has thus led to the demonstration that long-term continuous administration of PDE 5 inhibitors counteracts the development of a PD-like fibrotic plaque in a rat animal model of PD, later extended to the prevention of corporal fibrosis in rat models of erectile dysfunction. The experimental evidence for this novel therapeutical modality is discussed.

**Summary of paper P-8. The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes.**

**Introduction.** Endogenously-elicited iNOS induction counteracts fibrosis and oxidative stress in penile tissues in rat models of Peyronie's disease and erectile dysfunction.

**Aim.** The current study aimed to determine whether the genetic blockade of iNOS expression in the iNOS knock out (**iNOS KO**) mouse intensifies fibrosis and oxidative stress in the penile corpora cavernosa, and this is exacerbated by streptozotocin (**STZ**)-induced diabetes and counteracted by insulin.

**Main outcomes.** Quantitative assessment of histological and biochemical markers in mouse corporal tissue.

**Methods.** Male iNOS KO and wild type (**WT**) mice were left untreated or injected with STZ, with or without insulin treatment. At 8 weeks, glycemia, glucosuria and proteinuria were determined, and corporal tissue sections were obtained and subjected to Masson trichrome staining for smooth muscle (**SM**)/collagen ratio, and immunostaining for  $\alpha$ -smooth muscle actin (**ASMA**) for SM content, proliferating cell nuclear antigen (**PCNA**) for cell replication, TGF $\beta$ 1 as profibrotic factor, TUNEL assay for apoptosis, and xanthine oxidoreductase (**XOR**) for oxidative stress. Collagen was estimated by the hydroxyproline reaction.

**Results.** The corporal SM/collagen ratio and SM content were reduced, and collagen content increased in iNOS KO mice as compared to WT mice, but apoptosis was decreased and cell replication increased, whereas TGF $\beta$ 1 and XOR did not vary. Severe hyperglycemia caused in the WT a reduction of the corporal SM/collagen ratio and SM content, and an increase in apoptosis without changes in PCNA, TGF $\beta$ 1, or XOR. In the iNOS KO mouse the hyperglycemia-induced alterations were exacerbated, with additional increases in oxidative stress and TGF $\beta$ 1. Insulin normalized glycemia and partially protected the SM in both the WT and the iNOS KO mice.

**Conclusions.** The antifibrotic, antioxidative, and SM-protective roles of iNOS in the penile corpora cavernosa were confirmed in the iNOS KO/STZ mouse model. These findings support



the importance of endogenously-elicited iNOS induction in protecting the penile corpora cavernosa from the pro-fibrotic effects of hyperglycemia.

## **B. Pending experiments**

We aim to complete during the one-year no-cost extension the Masson trichrome staining for the smooth muscle/collagen ratio for group 2, and the hydroxyproline estimations for collagen content in groups 2-8, to determine whether despite the smooth muscle content appears essentially unchanged by the lower doses of sildenafil, collagen is in fact decreased as expected from the cavernosometry determinations.

We will also estimate the potential regeneration of nitrergic nerves by immunohistochemistry or immunofluorescence of corporal neurofilaments, by antibodies against synaptophysin or neurofilament-70 (NF-70), combined with nNOS estimation by quantitative image analysis.

Since the cavernosometry is virtually complete, we will also perform Masson trichrome and immunohistochemistry for ASMA for the two groups receiving MDSC supplemented or not with low sildenafil

If collagen is not affected by sildenafil at low doses (with or without molsidomine) and if time and resources allows it, we will investigate a complementary hypothesis not planned initially. This is whether, in addition to the already demonstrated antifibrotic and protective effects on the corporal smooth muscle exerted by the long-term sustained administration of high doses of sildenafil, there is a secondary site of action that can be detected at low doses when the effects on the corporal smooth muscle would be negligible. This will involve investigating one or several of the other potential sites of action of long-term lower doses of sildenafil: a) nitric oxide bioavailability in the corpora cavernosa, by increasing eNOS and nNOS content, that by producing more nitric oxide and cGMP, would synergize the reduction of XOR, and hence of ROS, already demonstrated; we may also estimate nitrotyrosine and NADPH oxidase; or b) the potential reduction of corporal tone, caused by a decrease in the expression of the rho-kinase system.

## **Bulleted list of key research accomplishments**

We have demonstrated in a rat model of erectile dysfunction subsequent to cavernosal nerve damage during radical prostatectomy for prostate cancer (bilateral cavernosal nerve resection in the rat, or BCNR), that:

- The improvement by continuous long-term oral administration of PDE5 inhibitors of the corpora cavernosal histopathology underlying the main cause of erectile dysfunction, CVOD, resulting from cavernosal nerve resection, is dose-dependent. We found that 2- and 8-fold dose decreases in the previously used dose of sildenafil (20 mg/kg/day), ameliorated the corresponding CVOD, even by a discontinuous but daily administration that mimics a daily pill regimen in men.
- However, these lower doses of sildenafil failed to counteract the smooth muscle loss or stimulate cell proliferation at the levels previously seen with higher doses.
- The long-term sustained intraperitoneal administration of a long-action nitric oxide donor, molsidomine, did not seem to be superior to sildenafil for correcting CVOD, and did not improve or potentiate its effects on the underlying histopathology. This, despite molsidomine was effective in protecting the corporal smooth muscle and particularly ameliorating oxidative stress in a condition, type 1 diabetes, that induces erectile dysfunction and a corporal histopathology similar to the one caused by BCNR. In fact, molsidomine also ameliorated oxidative stress in the BCNR model.

- The current results raise the question on whether additional mechanisms to the counteraction of corporal fibrosis and smooth muscle loss underlie the correction of CVOD subsequent to cavernosal nerve damage, induced by PDE5 inhibitors, and which is the key mechanism in this process. These topics are being approached during the no-cost extension, addressing particularly the potential regeneration by sildenafil of nerve terminals in the corpora and the prevention of fibrosis and loss of smooth muscle in the penile arteries.
- Another ongoing experiment where MDSC have been given in combination with sildenafil at the lowest dose, or alone, to BCNR rats has been successfully completed and a series of assays will determine during the no-cost extension whether sildenafil potentiates the putative beneficial effects of the stem cells on CVOD and the underlying histopathology.
- Our previous results reported in papers 1-6, and now in papers 7 and 8, have contributed to an intensive discussion among physicians involved in the treatment of erectile dysfunction, and particularly subsequent to radical prostatectomy after prostate cancer, about the possible switch of the "on demand", sporadic administration of PDE5 inhibitors, to a daily long-term regimen. This may become standard practice to prevent erectile dysfunction and even **LUTS** (lower urinary tract symptoms) in "penile rehabilitation" strategies after radical prostatectomy for prostate cancer. FDA has granted approval for tadalafil daily use, and several trials are ongoing to evaluate this treatment modality. A similar approach is being considered for another fibrotic condition in the penis: Peyronie's disease.

## Reportable outcomes for Year 2

Papers and abstracts follow a correlative numbering with those previously reported with the reports for Years 1 and 2.

### Papers acknowledging this grant PC061300 (W81XWH-07-1-0129) (see Appendix)

P7: **Gonzalez-Cadavid NF**, Rajfer J. Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. Nat Rev Urol. 2010 Apr;7(4):215-21. Epub 2010 Mar 9. PubMed PMID: 20212516.

P8. Ferrini MG, Moon J, Rivera S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF** (2010) The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. J Sex Med, in press

P9. Ferrini MG, Moon J, Rivera S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF**. Antifibrotic and antioxidant therapies prevent the development of fibrosis in the penile corpora cavernosa associated with type 1 diabetes in the iNOS knock out mouse. BJU Internat, 2010, to be submitted by 06/30.

Because of the need to complete the histopathological evaluation, we have not submitted additional papers other than the ones above and the six others related to this grant cited in the previous two reports. A new paper on BCNR is in preparation (P-10) and we expect to submit and include it in the final report

### Abstracts and presentations related to this grant (penile fibrosis and CVOD)

A9. Vernet D, Heydarkhan S, Kovanecz I, Lue Y-H, Rajfer J, **Gonzalez-Cadavid NF** (2009). Characterization of endogenous stem cells from the mouse penis that express an embryonic stem cell gene and undergo differentiation into several cell lineages. Am Urol Assoc Meet, Chicago, IL, J Urol,

For the same reasons as above no abstracts, additional to the one above and the other eight abstracts already reported, have been presented. Several new abstracts will be presented during the no-cost extension in the SMSNA and AUA meetings, as well as in the 2nd IMPaCT Conference to be held in Orlando, FL, March 9-12th, 2011

New applications for funding derived from preliminary results of this grant (penile fibrosis and CVOD)

The following grant applications have been submitted by investigators in this DOD grant using in part results obtained during year 1 of this grant.

1. Not funded: 1RC1DK086865-01 (PI: Gonzalez-Cadavid). Modulation of human iPS differentiation in radical prostatectomy-related erectile dysfunction in rat models. NIH Recovery Challenge Grants. 09/09-08/11. No overlapping. Scored 35 in the 5 percentile and was considered for funding but finally was not awarded. It was condensed into the grant below

2. To be resubmitted. NIH 1R21DK089996-01 (PI: Gonzalez-Cadavid) Human iPS in erectile dysfunction after radical prostatectomy in rat models 07/10-06/12. No overlapping. Scored 30 and was considered for funding but finally was not awarded.

3. Pending. NIH R21ES019465-01 (PI: Gonzalez-Cadavid) Bisphenol A effects on the peripheral mechanisms of penile erection 07/10-06/12. No overlapping. Scored 30 and still has good chances of funding

4. Pending. Animal Models of Diabetes Complication Consortium (PI: Gonzalez-Cadavid) A diabetes mouse model for studying endogenous/exogenous stem cell interaction. 08/10-07/11. No overlapping.

5. To be resubmitted. PI: Gonzalez-Cadavid NF). Erectile Dysfunction and Nitric Oxide Synthase in Aging. RO1 DK53069-08 (resubmission). 11/09-10/14. No overlapping. Scored 52 in the 49 percentile.

There are other four unrelated applications.

#### Appointments

None.

#### Conclusions

During years 1 and 2 we have shown that long-term sustained administration of high doses of PDE5 inhibitors (tadalafil and sildenafil, previously vardenafil), prevented in a rat model of cavernosal nerve damage post-radical prostatectomy the erectile dysfunction (corporal veno-occlusive dysfunction or CVOD) and the underlying penile corporal histopathology resulting from this surgery for prostate cancer in men.

Now, we have shown that much lower doses of sildenafil, combined or not with a nitric oxide donor, molsidomine, also correct the neuropraxia-induced CVOD, although so far the stimulation of smooth muscle repair and decrease of fibrosis in the corpora cavernosa do not seem to occur (ongoing assays). We have also studied in this model the effect of implanting MDSC into the corpora in the presence or absence of long-term sustained low dose sildenafil (ongoing).

During the no-cost extension we aim to complete these studies and determine whether sildenafil stimulates nerve terminal regeneration in the corpora, and, if possible, examine a complementary hypothesis to explain the correction of CVOD by low doses of sildenafil based on secondary tissue targets, such as NO bioavailability or rhokinase reduction.

Our results have pioneered and provided a scientific justification for the emerging shift from the current “on demand/sporadic” therapy of post-radical prostatectomy patients with PDE5 inhibitors, towards their long-term daily administration in the novel “penile preservation post-radical prostatectomy” clinical approach.

### **References**

They are listed in the papers enclosed in the Appendix

### **Appendices**

They include:

- 1) The downloaded publication for paper P-7
- 2) The proof for paper P-8
- 3) The biographical sketches of Drs. Gonzalez-Cadavid, Kovanecz, Vernet, Nolzco, and Rajfer

# Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy

Nestor F. Gonzalez-Cadavid and Jacob Rajfer

**Abstract** | Peyronie's disease (PD) is a localized fibrotic condition of the tunica albuginea that is associated with risk factors for corpora cavernosa fibrosis (such as advanced age and diabetes) and Dupuytren contracture, another localized fibrotic process. Most of the current pharmacological treatments for PD are not based on antifibrotic approaches that have shown promising results in animal models and clinical efficacy in other fibrotic conditions, which may explain why they are generally unsuccessful. Evidence gathered in human specimens and animal models of PD have elucidated aspects of its etiology and histopathology, showing that overexpression of transforming growth factor  $\beta$ 1, plasminogen activator inhibitor 1, reactive oxygen species and other profibrotic factors, which are, in most cases, assumed to be induced by trauma to the tunica albuginea, leads to myofibroblast accumulation and excessive deposition of collagen. At the same time, a steady overexpression of inducible nitric oxide synthase, leading to increased nitric oxide and cGMP levels, seems to act as an endogenous antifibrotic mechanism. This process has also been reported in corporal and cardiovascular fibrosis, and has led to the demonstration that long-term continuous administration of phosphodiesterase type 5 inhibitors counteracts the development of a PD-like fibrotic plaque in a rat model, and later extended to the prevention of corporal fibrosis in animal models of erectile dysfunction.

Gonzalez-Cadavid, N. F. & Rajfer, J. *Nat. Rev. Urol.* advance online publication 9 March 2010; doi:10.1038/nrurol.2010.24

## Introduction

Peyronie's disease (PD) is somewhat of a misnomer, as it is actually restricted to a small localized fibrotic plaque of the tunica albuginea of the penis.<sup>1,2</sup> Epidemiologic studies suggest that the disease may be present in up to 10% of all men, but primarily affects those in their sixties and seventies.<sup>1–6</sup> The reason PD attracts attention is that many men with the disease have some form of erectile dysfunction, and in the erect state the afflicted organ tends to curve and may be painful during intercourse. Despite its typical fibrotic histopathology, this condition is not associated with other localized or diffuse fibrotic processes, with the single exception of Dupuytren contraction, with which it shares a similar histopathology.<sup>7–9</sup> No satisfactory medical treatments for PD are currently available; however, experimental models have provided new insights into its pathophysiology and etiology, which have facilitated the investigation of alternative therapeutic approaches, including long-term continuous administration of phosphodiesterase type 5 (PDE5) inhibitors<sup>10</sup> as an antifibrotic modality. In this Review we examine the experimental evidence that forms the basis for this treatment strategy. No reports of the clinical efficacy of long-term PDE5 inhibition in patients with PD have been published, and, although the first preliminary animal study dates back to 2003,<sup>11</sup> this should still be considered as a novel management approach that requires future clinical validation.

## Competing interests

The authors declare no competing interests.

## Pathophysiology

A widely accepted hypothesis on the etiology of the PD plaque is that it originates from trauma or microtrauma to the erect penis, primarily during different types of sexual activities.<sup>12</sup> This hypothesis is based mainly on the demonstration of fibrin staining or immunodetection in tissue sections of human PD plaques,<sup>13–15</sup> findings that have been corroborated in an animal model of PD.<sup>14</sup> A plausible interpretation of this hypothesis is that the fibrin originates from fibrinogen that has extravasated into the interstices of the tunica albuginea during a traumatic sexual episode. Inhibition of the fibrinolytic system or an inability to degrade the intravasated fibrin would then lead to its persistence in the tunica, which initially leads to an acute inflammatory response. Because the fibrin is not degraded, the protein continues to exert a proinflammatory response, which ultimately leads to an abnormal healing process. The end result is the formation of a 'scar' that at some time evolves into a palpable plaque.<sup>16</sup> Indeed, injection of fibrin directly into the tunica albuginea of the rat penis elicits a PD-like plaque, resembling in many aspects the histology and evolution of the human PD plaque.<sup>14,15,17</sup>

The epidemiological association of PD with a history of sexually elicited trauma of the penile or pelvic surgery, which may affect the homeostasis of tissues in the penis, supports the trauma-related hypothesis for at least part of the patient population,<sup>8,18,19</sup> despite some contradictory evidence.<sup>5,8,20</sup> Considering the association of PD with Dupuytren contracture, genetic or immune-related predisposition to PD may modulate the tunical healing

Department of Urology, David Geffen School of Medicine at UCLA, and Los Angeles Biomedical Research Institute (LABioMed) at Harbor-UCLA Medical Center, Building F-6, 1124 West Carson Street, Torrance, CA 90502, USA (N. F. Gonzalez-Cadavid, J. Rajfer).

Correspondence to: N. F. Gonzalez-Cadavid [ncadavid@ucla.edu](mailto:ncadavid@ucla.edu)

## Key points

- Rodent models of Peyronie's disease (PD) are representative of most of the main histological and biochemical features present in human specimens
- Cell cultures obtained from the human PD plaque and its rat counterpart have added to the experimental evidence acquired in the human and in animal models
- Endogenous mechanisms of defense against tunical tissue inflammation, oxidative stress and fibrosis have been detected in the PD plaque and the rat PD-like lesion, and may be mimicked pharmacologically for treatment
- Endogenously elicited inducible nitric oxide synthase leads to sustained production of nitric oxide and cGMP, which counteract myofibroblast differentiation, accumulation of reactive oxygen species, cytokine release, and collagen deposition
- Continuous long-term administration of nitric oxide donors and phosphodiesterase 5 inhibitors has shown preventive and corrective effects in a rat model of PD: studies in patients with PD are now needed

reaction following any type of trauma to the penis, but this possibility has not been studied as intensively as is needed.<sup>21–23</sup>

The pathology of the PD plaque has been investigated in a variety of studies in human and animal model specimens and in related cell cultures.<sup>1,10</sup> Based on the results of histochemistry, immunohistochemistry and other assays performed on the human PD plaque tissues, it is clear that fibrosis—the excessive deposition of collagen and extracellular matrix (ECM) with disorganization of collagen fibers and loss of elastic fibers—is the main pathological process, combined in most cases with fibrin accumulation and different degrees of inflammation.<sup>12–14,24,25</sup>

Myofibroblasts (cells that share the fibroblast and smooth muscle phenotypes<sup>26,27</sup>) are not normally present in the tunica albuginea of the penis, but have been identified as the cells responsible for the disarrangement of the ECM in the PD plaque.<sup>28–32</sup> The normal process of apoptosis that eliminates myofibroblasts after they have fulfilled their role in wound healing is somehow inhibited in PD, thus leading to their persistence in the tunica albuginea. This myofibroblast accumulation is common not just to scar formation in the skin or the infarcted heart, but to most other types of fibrosis.<sup>26,27</sup> The PD plaque becomes harder by progressing through an intensification of fibrosis (with or without the persistence of inflammation) and, in at least 15% of the patients, through an advanced stage of calcification and ossification involving osteoblasts.<sup>33,34</sup> Spontaneous regression of the plaque after its initial formation occurs in rare cases.<sup>9,34</sup>

The scattered evidence regarding human PD plaque tissues has been considerably expanded by systematic approaches in experimental animal models, mainly in the widely used rat model of PD induced by transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1).<sup>1,10</sup> This key profibrotic factor, present in multiple tissues<sup>35</sup> and produced in the human PD plaque, is found at increased levels in the blood of PD patients.<sup>36</sup> Similarly, when a peptide derived from the TGF- $\beta$ 1 sequence is injected into the rat tunica, a plaque resembling that seen in human PD is found around

45 days later<sup>11,37–41</sup> at the injection site. Other less frequently employed but nonetheless useful animal models are based on either the successive injections of an adenoviral construct expressing a constitutively active TGF- $\beta$ 1 protein, leading to penile curvature during the erect state and, at times, calcification within the plaque,<sup>42</sup> or on a single fibrin injection that mimics the extravasation of fibrinogen, initiating acute inflammation followed by the rapid development of the PD-like plaque.<sup>14,15,17</sup> Both TGF- $\beta$ 1 and myostatin, another profibrotic factor within the TGF- $\beta$  family, are involved in this process,<sup>41</sup> and it is quite likely that the key downstream signaling occurs via the Smad pathway, which is the mechanism common to most factors in this family.<sup>43</sup> A tight skin (Tsk) mouse model has been described that develops a spontaneous PD-like plaque with penile bending and areas of chondroid metaplasia with heterotypic ossification.<sup>44</sup>

These animal models, therefore, represent most of the histologic and biochemical features of the human PD plaque, including inflammation, myofibroblast accumulation, collagen deposition, oxidative stress, calcification, ossification and penile bending, among others. Thus, we believe that the PD-like lesion, either elicited experimentally or by spontaneous mutations in the rodent tunica, is more complex than a mere tunical fibrosis event, and, imperfect as most disease models in laboratory animals are, this experimental plaque is adequate for preclinical testing of various therapeutic strategies for PD.

Finally, the use of cell cultures from the normal, myofibroblast-free tunica albuginea or from the human PD plaque or the induced PD-like plaque from a rat, which are enriched in myofibroblasts, has allowed us to more precisely define the role of myofibroblasts in the pathophysiology of PD.<sup>28–32,45,46</sup> These cells have been shown to be responsible for the excessive collagen deposition seen in PD, and have even been postulated to cause penile bending by their contractile features. Moreover, pluripotent stem cells have been identified in the PD cultures. This may explain the fibrotic and osteogenic progression of the PD plaque upon the release of cytokines following microtrauma to the penis, which would stimulate stem cell commitment to this cell lineage.<sup>31,45</sup> PD fibroblasts are also potentially tumorigenic, or acquire this trait upon culture, but it is not known whether this is related to the presence of stem cells.<sup>47</sup>

There is no doubt that cell cultures derived from the human PD plaque and normal tunica albuginea closely represent their respective histologic features, notwithstanding the obvious shortcomings of any type of cell culture compared to the *in vivo* tissue. This has been tested with a multiplicity of immunocytochemical and western blot markers, as well as DNA microarrays and reverse transcription polymerase chain reaction procedures for the detection of fibroblasts, myofibroblasts and stem cells, and their respective differentiation and roles in inflammatory and fibrotic processes. The situation is similar regarding cell cultures obtained from the rat PD-like plaque, which have been shown to mimic their human counterparts. All these cultures have been useful

tools for defining therapeutic targets at the cellular and molecular level.

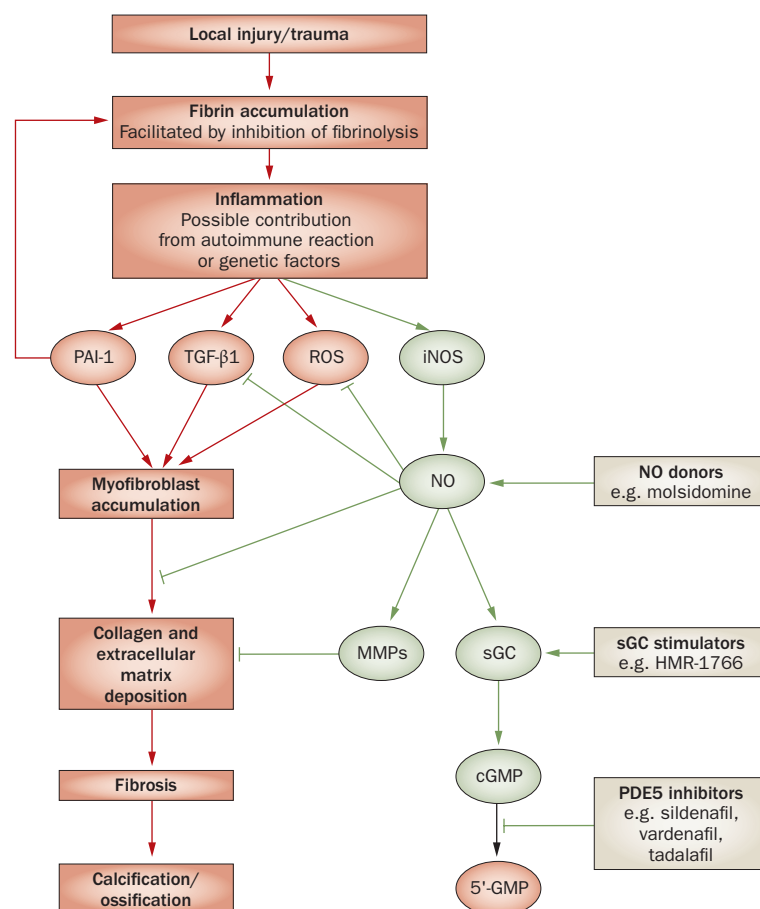
### Cellular and molecular mechanisms

Results from experimental studies that have employed a variety of cellular and molecular biology techniques in the PD models described above, combined with the information obtained from the analysis of the human PD plaque, have made it possible to define an overall mechanistic picture of the initiation and progression of the PD plaque.<sup>48</sup> The mechanism resembles that seen in some other localized fibroses, including the more gradual and diffuse type that occurs in the penile corpora cavernosa of men with erectile dysfunction and many animal models of this disorder.<sup>49</sup> The main features of PD fibrosis are described below.

Fibrinogen extravasated into the tunica albuginea of the penis accumulates at the site of the future PD plaque owing to inhibition of the fibrinolytic and other proteolytic systems, primarily due to overexpression of plasminogen activator inhibitor 1 (PAI-1). The resulting fibrin formation, and possibly with the assistance of immunoglobulins and other extravasated proteins, triggers the release and/or activation of TGF- $\beta$ 1, PAI-1, and reactive oxygen species (ROS), which are recognized as key profibrotic factors in many tissues, including the kidney and vascular system. Concurrent expression of other cytokines, including monocyte chemoattractant protein 1 (MCP-1; also known as CC-chemokine ligand 2 [CCL-2]), which is associated with acute inflammation that often progresses to a chronic phase, overexpression of other members of the TGF- $\beta$ 1 family (such as myostatin) and components of their common Smad signaling pathway, and other unknown agents combine to elicit the fibrotic process. The PD plaque then develops through excessive collagen deposition, elastin degradation, myofibroblast differentiation from fibroblasts or stem cells in the tunica, oxidative stress, and eventually calcification (Figure 1).<sup>10,48,50,51</sup>

The accumulation of tissue inhibitors of metalloproteinases (TIMPs) and the relative inhibition of collagenases (and/or a possible downregulation of their expression), which interferes with the normal breakdown of the accumulated collagen—and potentially, in the case of TIMPs, with therapeutic collagenase delivered to the plaque—contribute to the maintenance of the fibrotic process.<sup>52</sup>

One of the main findings stemming from DNA microarray analysis of the molecular profile of the PD plaque is the recognition that this tissue may be undergoing constant cellular and molecular turnover, and that spontaneous development of defense mechanisms to counteract fibrosis and oxidative stress might occur.<sup>53,54</sup> This transcriptional analysis detected overproduction of matrix metalloproteinases (MMPs) 2 and 9 (which contribute to collagen breakdown), decorin (which binds and neutralizes TGF- $\beta$ 1), and thymosins (which activate MMPs) in both PD plaques and Dupuytren nodules, as well as in cell cultures of these tissues. All these proteins seem to act as antifibrotic agents, either by combating



**Figure 1** | Schematic representation of the etiology and pathophysiology of the fibrotic process leading to the Peyronie disease plaque (red), and the antifibrotic effects (green) of endogenous iNOS. Fibrin extravasated after injury or trauma to the penis accumulates in the tunica albuginea, which induces a local proinflammatory response. This leads to increased production of several key profibrotic factors, including PAI-1 (which inhibits fibrinolysis), TGF- $\beta$ 1 and ROS, which contribute to the accumulation of myofibroblasts. Myofibroblasts are responsible for the increased collagen and extracellular matrix deposition leading to fibrosis, and, owing to inhibited apoptosis of these cells, persist in the tunica albuginea. The production of iNOS seems to be an endogenous antifibrotic factor, leading to increased levels of NO in the affected tissue. NO inhibits collagen synthesis and myofibroblast differentiation and reduces oxidative stress by quenching ROS. In addition, NO increases the activity of sGC, which in turn increases levels of cGMP which has further antifibrotic effects. Potential pharmacological interventions (gold) that might prevent or partially reverse plaque formation include NO donors, sGC stimulators and PDE5 inhibitors. Abbreviations: iNOS, inducible nitric oxide synthase; MMPs, matrix metalloproteinases; NO, nitric oxide; PAI-1, plasminogen activator inhibitor 1; PDE5, phosphodiesterase type 5; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; TGF- $\beta$ 1, transforming growth factor  $\beta$ 1.

collagen deposition or promoting its breakdown; therefore, it is plausible to postulate that they are produced in response to the fibrotic processes and that progression depends on the balance between the noxious and protective mechanisms, which in some cases may lead to spontaneous regression of the plaque.

### Role of inducible nitric oxide synthase

Despite the experimental evidence outlined above, the current pharmacological management of PD is mostly



empirical, as it is generally based on the use of drugs targeting nonspecific or ancillary aspects of PD, such as inflammation or cell replication.<sup>2,55</sup> Virtually nothing has been translated from the abundant pharmacological studies in other types of fibrosis, or from pre-clinical studies in animal or cell culture models of PD. This renders PD a sort of orphan disease in terms of a scientifically rational approach to therapy, in contrast to the other types of fibrosis where, in general, clinical use is supported by promising preclinical studies.<sup>48,56</sup>

However, among the putative endogenous mechanisms of defense against fibrosis that are postulated to operate in the PD plaque, the most intensively studied and highly promising in terms of therapeutic potential is the spontaneous induction of inducible nitric oxide synthase (iNOS), a NOS isoform that is not expressed in normal penile tissue.<sup>57</sup> Whereas in the past it was assumed that the presence of iNOS portended a deleterious outcome to a tissue, it is now believed to in fact be a protective mechanism against tissue fibrosis in certain settings. iNOS expression produces a steady output of nitric oxide, a compound that directly inhibits collagen synthesis and myofibroblast differentiation, quenches ROS via the production of peroxynitrite, and inhibits the TGF- $\beta$ /Smad signaling pathway, thus counteracting fibrosis.<sup>11,17,25,30,41</sup> The pro-apoptotic effects of nitric oxide might also contribute to reducing the myofibroblast population. Remarkably, iNOS production in the penis is not restricted to the tunica albuginea or to PD. For example, iNOS is detectable in the corpora cavernosa of patients with diabetes, advanced age, and even following radical prostatectomy, where fibrosis of the corpora leads to the development of corporal veno-occlusive dysfunction (CVOD). iNOS production may also been seen in the penile arteries in disease states where arteriosclerosis or arterial stiffness is present.<sup>57–64</sup> In all these scenarios, iNOS production, resulting in some cases from an inflammatory process, is presumed to be an antifibrotic response to the development of fibrosis within these individual tissues.

Collectively, several lines of evidence in rodent models support the antifibrotic role of iNOS in the PD plaque and in corporal and vascular tissue. Gene transfer of iNOS cDNA, which then becomes constitutively expressed, reduces fibrosis in the tunica albuginea and corpora of the penis, whereas long-term, specific inhibition of iNOS activity (by the iNOS inhibitor L-NIL) counteracts this process in both tissues, as well as in the arterial wall.<sup>17,25,58,60</sup> Furthermore, genetic inactivation of the iNOS gene in the iNOS knockout mouse intensifies collagen deposition in the corpora in a process exacerbated by diabetes.<sup>64</sup> This agrees with what has been shown in other tissues in the iNOS knockout mouse model, in which the absence of iNOS increases interstitial fibrosis after unilateral ureteral obstruction and hepatic fibrosis in animals fed a high-fat diet and in those with streptozotocin-induced diabetic nephropathy.<sup>65–70</sup> iNOS also has a cardioprotective role in preconditioning during ischemia reperfusion injury in mouse kidney and in granulomatous disease.<sup>71,72</sup>

Some evidence suggests that iNOS deletion seems to be protective rather than detrimental in certain types of fibrosis,<sup>73–76</sup> and that iNOS overexpression is associated with increased fibrosis, particularly in the diabetic kidney.<sup>77</sup> However, nitric oxide is known to inhibit myofibroblast differentiation via inhibition of the TGF- $\beta$ /Smad pathway, and to have general antifibrotic effects via inhibition of collagen synthesis and ROS quenching.<sup>78–82</sup> The protective effects of iNOS depend, therefore, on the specific tissue type and the pathological conditions under which it is induced. In the case of the penile tissues, including the PD plaque, all the evidence so far obtained supports an antifibrotic role for iNOS.

### Treatment with PDE5 inhibitors

Although some of the beneficial, antifibrotic effects of iNOS are directly attributable to nitric oxide, others may result from the increased levels of cGMP produced following stimulation of guanylate cyclase by nitric oxide, which subsequently leads to protein kinase G activation. cGMP, and in some cases PDE5 inhibitors, have been shown to inhibit myofibroblast formation in cell cultures of human and rat PD plaques<sup>30,32</sup> and in lung fibroblasts.<sup>83</sup> These antifibrotic effects are also exercised by guanylate cyclase stimulators via protein kinase G stimulation and inhibition of fibrotic mediators such as angiotensin II, or by TGF- $\beta$  or Rho activation.<sup>83–87</sup>

An early preliminary study in the rat model of TGF- $\beta$ 1-induced PD demonstrated that both oral sildenafil, a PDE5 inhibitor that protects cGMP from breakdown, and oral pentoxifylline, a predominantly PDE4 inhibitor that increases cAMP synthesis, counteract the development of the PD-like plaque.<sup>11</sup> In the case of pentoxifylline, it was proposed that the well-known cAMP–cGMP signaling crosstalk may be responsible for its antifibrotic effects, although direct effects of cAMP or the involvement of alternative pathways modulated by pentoxifylline can not be excluded. This study revealed a completely new mechanism of action for PDE5 inhibitors, in contrast to their standard on-demand clinical administration to facilitate penile erection upon sexual stimulation, which is mediated by their short-term relaxant effect on the corporal and arterial smooth muscle produced by a transient elevation of cGMP levels. The novel concept is that PDE5 inhibitors given for a sufficiently long time can induce a sustained elevation of nitric oxide and cGMP levels that, independently of their vasorelaxant effects, which would show only during sexual stimulation, act as antifibrotic agents by reducing collagen deposition, profibrotic factor release, oxidative stress and myofibroblast numbers.

In a subsequent study in the same rat model, it was shown that another PDE5 inhibitor, vardenafil, given orally and in different dosing regimens, not only prevented but partially reversed the formation of the PD-like plaque.<sup>40</sup> To test the early preventive effects of vardenafil, the drug was administered to male rats either in their drinking water or as a once-daily oral instillation at either 1 or 3 mg/kg per day for 45 days following a single injection of TGF- $\beta$ 1 into the tunica albuginea



to induce the PD-like plaque. Other animals, in which a PD-like plaque had already been formed, received either dose of vardenafil in their drinking water for 42 days (late, therapeutic administration). Preventive treatment at the higher dose (both continuous and once-daily treatments) reduced the overall collagen content, collagen III/I ratio and the number of myofibroblasts and TGF- $\beta$ 1-positive cells, and selectively increased the apoptotic index of cells (presumably including myofibroblasts), in the PD-like plaque. The lower dose was less effective. When vardenafil was given continuously in the drinking water for 42 days after the PD-like plaque was formed, a partial reduction in plaque size was observed. From these two studies,<sup>11,40</sup> it was concluded that long-term oral treatment with a PDE5 inhibitor slows and reverses the early stages of an experimental PD-like plaque in the rat, and might ameliorate a more advanced plaque.

The optimal therapeutic regimen for discontinuous oral administration of PDE5 inhibitors was not assessed in these studies, so whether oral instillation, perhaps at a higher dose, can regress an already formed plaque is not known. However, the authors discussed the possibility of testing combinations of PDE5 inhibitors and other compounds used for the treatment of PD, such as verapamil (a calcium channel blocker), vitamin E (an antioxidant) and collagenase. An important point that was made was that, owing to the multifactorial nature of fibrosis and the difficulty of reversing established collagen crosslinking, combination therapy might be more effective than a single agent when a well-formed PD plaque is present.<sup>40</sup>

This first demonstration of the antifibrotic effects of long-term, continuous administration of a PDE5 inhibitor was later extended to the corpora cavernosal fibrosis that underlies CVOD, caused either by aging or by neuropraxia secondary to cavernosal nerve resection, mimicking the post-radical-prostatectomy state.<sup>59–62</sup> In these cases, the effects of the three PDE5 inhibitors (sildenafil, vardenafil and tadalafil) on collagen deposition in the rat corpora were similar to those seen in the PD-like plaque; however, they also seemed to provide protection against the loss of smooth muscle cells, which are responsible for normal corporal compliance and their ability to relax and achieve normal veno-occlusion. In fact, the PDE5 inhibitors decreased corporal apoptosis—specifically of smooth muscle cells in this case, as opposed to the increased apoptotic index in tunical myofibroblasts observed in the PD plaque—and oxidative stress, thus preventing or correcting CVOD. Sildenafil prevented the progression of corporal fibrosis in penile histopathology induced by cavernous neurotomy in the rat and in patients who had undergone radical prostatectomy.<sup>88–90</sup> These antifibrotic effects of PDE5 inhibitors, specifically the prevention of collagen deposition and the inhibition of TGF- $\beta$ 1 expression and oxidative stress, were also seen in rat models of diabetic nephropathy, experimental glomerulonephritis, myocardial infarction and hypertrophy, and pulmonary fibrosis;<sup>91–95</sup> therefore, their antifibrotic effects do not seem to be restricted to

penile tissues. These effects should not be confused with the beneficial vasodilator mechanism exploited for the treatment of pulmonary hypertension.<sup>96</sup>

Despite the two experimental papers on the effects of continuous long-term treatment with sildenafil and vardenafil on the PD-like plaque in the TGF- $\beta$ 1 rat model,<sup>11,40</sup> the emerging literature on this modality in other types of tissue fibrosis, and the well characterized antifibrotic effects of cGMP and guanylate cyclase stimulators, no similar experimental studies have been performed in human patients with PD. An article related to the use of PDE5 inhibitors in patients with PD in fact focused on their standard “on-demand” application for treating erectile dysfunction, and not PD itself.<sup>97</sup> This lack of studies in humans does not seem to be due to concerns about potential adverse effects, as several trials have shown that daily administration of sildenafil or tadalafil is well tolerated.<sup>98,99</sup> Moreover, a 2006 case report described the beneficial effects of an antifibrotic regimen of drugs that upregulate nitric oxide (and, therefore, cGMP production) in two patients with refractory priapism (>48 h duration).<sup>43</sup> Based on the previous work in a rat model of PD,<sup>11</sup> the regimen included the PDE inhibitors pentoxifylline and sildenafil and the nitric oxide precursor L-arginine. At 1 year, both patients were found to have flexible corpora and no evidence of fibrosis.

## Conclusions

Despite the strong preclinical evidence in animal models supporting the antifibrotic effects of continuous, long-term administration of PDE5 inhibitors in penile tissue, this approach has yet to be studied in patients with PD. The likelihood is that our wider experience of the on-demand use of PDE5 inhibitors for erectile dysfunction will eventually lead to the first clinical test of the antifibrotic hypothesis in the context of the relatively mild corporal fibrosis seen in patients after radical prostatectomy; only if successful in this application might its use be extended to PD. In any case, despite the promise of this novel approach, the progression of the human PD plaque to advanced fibrosis and calcification may restrict its application to the early stages of the disease. In addition, a combination regimen comprising PDE5 inhibitors and other agents that stimulate collagen breakdown may be needed to effectively reduce the size of an established plaque. We believe that a study in which the outcomes of men receiving a currently used treatment for PD plus a PDE5 inhibitor are compared with men receiving the same treatment plus placebo will help define the future role of PDE5 inhibitors in patients with PD.

## Review criteria

We searched for original articles focusing on Peyronie's disease in PubMed published from 1980 onwards. The search terms we used were “Peyronie's disease” and “La Peyronie”. All papers identified were full-text papers (unless indicated in the reference list) and were published in English, French or Spanish.

1. Gonzalez-Cadavid, N. F. & Rajfer, J. In *Current Clinical Urology: Peyronie's Disease, A Guide to Clinical Management* (ed. Levine, L. A.) 19–39 (Humana Press, Totowa, 2007).
2. Smith, J. F., Walsh, T. J. & Lue, T. F. Peyronie's disease: a critical appraisal of current diagnosis and treatment. *Int. J. Impot. Res.* **20**, 445–459 (2008).
3. Müller, A. & Mulhall, J. P. Peyronie's disease intervention trials: methodological challenges and issues. *J. Sex. Med.* **6**, 848–861 (2009).
4. Smith, C. J., McMahon, C. & Shabsigh, R. Peyronie's disease: the epidemiology, aetiology and clinical evaluation of deformity. *BJU Int.* **95**, 729–732 (2005).
5. Mulhall, J. P. *et al.* Subjective and objective analysis of the prevalence of Peyronie's disease in a population of men presenting for prostate cancer screening. *J. Urol.* **171**, 2350–2353 (2004).
6. Taylor, F. L. & Levine, L. A. Peyronie's disease. *Urol. Clin. North Am.* **34**, 517–534 (2007).
7. Carrieri, M. P., Serraino, D., Palmiotto, F., Nucci, G. & Sasso, F. A case–control study on risk factors for Peyronie's disease. *J. Clin. Epidemiol.* **51**, 511–515 (1998).
8. Deveci, S. *et al.* Defining the clinical characteristics of Peyronie's disease in young men. *J. Sex. Med.* **4**, 485–490 (2007).
9. Bjekic, M. D., Vlajinac, H. D., Sipetic, S. B. & Marinkovic, J. M. Risk factors for Peyronie's disease: a case–control study. *BJU Int.* **97**, 570–574 (2006).
10. Gonzalez-Cadavid, N. F. & Rajfer, J. Experimental models of Peyronie's disease. Implications for new therapies. *J. Sex. Med.* **6**, 303–313 (2009).
11. Valente, E. G. *et al.* L-Arginine and phosphodiesterase (PDE) inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. *Nitric Oxide* **9**, 229–244 (2003).
12. Devine, C. J. Jr, Somers, K. D., Jordan, S. G. & Schlossberg, S. M. Proposal: trauma as the cause of the Peyronie's lesion. *J. Urol.* **157**, 285–290 (1997).
13. Somers, K. D. & Dawson, D. M. Fibrin deposition in Peyronie's disease plaque. *J. Urol.* **157**, 311–315 (1997).
14. Davila, H. H., Magee, T. R., Zuniga, F. I., Rajfer, J. & Gonzalez-Cadavid, N. F. Peyronie's disease associated with increase in plasminogen activator inhibitor in fibrotic plaque. *Urology* **65**, 645–648 (2005).
15. Davila, H. H., Ferrini, M. G., Rajfer, J. & Gonzalez-Cadavid, N. F. Fibrin as an inducer of fibrosis in the tunica albuginea of the rat: a new animal model of Peyronie's disease. *BJU Int.* **91**, 830–838 (2003).
16. Ehrlich, H. P. Scar contracture: cellular and connective tissue aspects in Peyronie's disease. *J. Urol.* **157**, 316–319 (1997).
17. Davila, H. H., Magee, T. R., Vernet, D., Rajfer, J. & Gonzalez-Cadavid, N. F. Gene transfer of inducible nitric oxide synthase complementary DNA regresses the fibrotic plaque in an animal model of Peyronie's disease. *Biol. Reprod.* **71**, 1568–1577 (2004).
18. Usta, M. F. *et al.* Relationship between the severity of penile curvature and the presence of comorbidities in men with Peyronie's disease. *J. Urol.* **171**, 775–779 (2004).
19. Perimenis, P., Athanasopoulos, A., Gyftopoulos, K., Katsenis, G. & Barbaliadis, G. Peyronie's disease: epidemiology and clinical presentation of 134 cases. *Int. Urol. Nephrol.* **32**, 691–694 (2001).
20. El-Sakka, A. I., Selph, C. A., Yen, T. S., Dahiya, R. & Lue, T. F. The effect of surgical trauma on rat tunica albuginea. *J. Urol.* **159**, 1700–1707 (1998).
21. Schiavino, D. *et al.* Immunologic findings in Peyronie's disease: a controlled study. *Urology* **50**, 764–768 (1997).
22. Noss, M. B., Day, N. S., Christ, G. J. & Melman, A. The genetics and immunology of Peyronie's disease. *Int. J. Impot. Res.* **12** (Suppl. 4), S127–S132 (2000).
23. Hauck, E. W., Hauptmann, A., Weidner, W., Bein, G. & Hackstein, H. Prospective analysis of HLA classes I and II antigen frequency in patients with Peyronie's disease. *J. Urol.* **170**, 1443–1446 (2003).
24. Anafarta, K., Bedük, Y., Uluoglu, O., Aydos, K. & Baltaci, S. The significance of histopathological changes of the normal tunica albuginea in Peyronie's disease. *Int. Urol. Nephrol.* **26**, 71–77 (1994).
25. Ferrini, M. G. *et al.* Antifibrotic role of inducible nitric oxide synthase. *Nitric Oxide* **6**, 283–294 (2002).
26. Hinz, B. *et al.* The myofibroblast: one function, multiple origins. *Am. J. Pathol.* **170**, 1807–1816 (2007).
27. Eyden, B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. *J. Cell. Mol. Med.* **12**, 2022–2037 (2008).
28. Somers, K. D. *et al.* Cell culture of Peyronie's disease plaque and normal penile tissue. *J. Urol.* **127**, 585–588 (1982).
29. Mulhall, J. P., Anderson, M. S., Lubrano, T. & Shankey, T. V. Peyronie's disease cell culture models: phenotypic, genotypic and functional analyses. *Int. J. Impot. Res.* **14**, 397–405 (2002).
30. Vernet, D. *et al.* Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. *Nitric Oxide* **7**, 262–276 (2002).
31. Vernet, D. *et al.* Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for peyronie disease. *Biol. Reprod.* **73**, 1199–1210 (2005).
32. Vernet, D. *et al.* Phosphodiesterase type 5 is not upregulated by tadalafil in cultures of human penile cells. *J. Sex. Med.* **3**, 84–94 (2006).
33. Vande Berg, J. S. *et al.* Mechanisms of calcification in Peyronie's disease. *J. Urol.* **127**, 52–54 (1982).
34. Kadioglu, A. *et al.* A retrospective review of 307 men with Peyronie's disease. *J. Urol.* **168**, 1075–1079 (2002).
35. Wynn, T. A. Cellular and molecular mechanisms of fibrosis. *J. Pathol.* **214**, 199–210 (2008).
36. El-Sakka, A. I., Hassoba, H. M., Pillarisetty, R. J., Dahiya, R. & Lue, T. F. Peyronie's disease is associated with an increase in transforming growth factor-beta protein expression. *J. Urol.* **158**, 1391–1394 (1997).
37. El-Sakka, A. I. *et al.* An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. *J. Urol.* **158**, 2284–2290 (1997).
38. El-Sakka, A. I. *et al.* Histological and ultrastructural alterations in an animal model of Peyronie's disease. *Br. J. Urol.* **81**, 445–452 (1998).
39. Bivalacqua, T. J. *et al.* A rat model of Peyronie's disease associated with a decrease in erectile activity and an increase in inducible nitric oxide synthase protein expression. *J. Urol.* **163**, 1992–1998 (2000).
40. Ferrini, M. G., Kovancec, I., Nolzco, G., Rajfer, J. & Gonzalez-Cadavid, N. F. Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int.* **97**, 625–633 (2006).
41. Cantini, L. P. *et al.* Profibrotic role of myostatin in Peyronie's disease. *J. Sex. Med.* **5**, 1607–1622 (2008).
42. Piao, S. *et al.* Repeated intratunical injection of adenovirus expressing transforming growth factor- $\beta$ 1 in a rat induces penile curvature with tunical fibrotic plaque: a useful model for the study of Peyronie's disease. *Int. J. Androl.* **31**, 346–353 (2008).
43. Rajfer, J., Gore, J. L., Kaufman, J. & Gonzalez-Cadavid, N. Case report: avoidance of palpable corporal fibrosis due to priapism with upregulators of nitric oxide. *J. Sex. Med.* **3**, 173–176 (2006).
44. Lucattelli, M. *et al.* A new mouse model of Peyronie's disease: an increased expression of hypoxia-inducible factor-1 target genes during the development of penile changes. *Int. J. Biochem. Cell Biol.* **40**, 2638–2648 (2008).
45. Nolzco, G. *et al.* Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int.* **101**, 1156–1164 (2008).
46. Mulhall, J. P. Expanding the paradigm for plaque development in Peyronie's disease. *Int. J. Impot. Res.* **15** (Suppl. 5), S93–S102 (2003).
47. Mulhall, J. P. *et al.* Peyronie's disease fibroblasts demonstrate tumorigenicity in the severe combined immunodeficient (SCID) mouse model. *Int. J. Impot. Res.* **16**, 99–104 (2004).
48. Gonzalez-Cadavid, N. F. & Rajfer, J. Mechanisms of disease: new insights into the cellular and molecular pathology of Peyronie's disease. *Nat. Clin. Pract. Urol.* **2**, 291–297 (2005).
49. Gonzalez-Cadavid, N. F. Mechanisms of penile fibrosis. *J. Sex. Med.* **6** (Suppl. 3), 353–362 (2009).
50. Nehra, A. & Nylhall, J. Peyronie's disease. In *Standard Practice in Sexual Medicine* 1<sup>st</sup> edn (eds Porst, H. & Buvat, J.) 158–173 (Blackwell, Malden, 2006).
51. Mulhall, J. P. Expanding the paradigm for plaque development in Peyronie's disease. *Int. J. Impot. Res.* **15** (Suppl. 5), S93–S102 (2003).
52. Del Carlo, M., Cole, A. A. & Levine, L. A. Differential calcium independent regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by interleukin-1 $\beta$  and transforming growth factor- $\beta$  in Peyronie's plaque fibroblasts. *J. Urol.* **179**, 2447–2455 (2008).
53. Magee, T. R. *et al.* Gene expression profiles in the Peyronie's disease plaque. *Urology* **59**, 451–457 (2002).
54. Qian, A., Meals, R. A., Rajfer, J. & Gonzalez-Cadavid, N. F. Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. *Urology* **64**, 399–404 (2004).
55. Hellstrom, W. Medical management of Peyronie's disease. *J. Androl.* **30**, 397–404 (2009).
56. Sivakumar, P. & Das, A. M. Fibrosis, chronic inflammation and new pathways for drug discovery. *Inflamm. Res.* **57**, 410–418 (2008).
57. Gonzalez-Cadavid, N. F. & Rajfer, J. The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. *Curr. Pharm. Des.* **11**, 4041–4046 (2005).
58. Ferrini, M. G., Davila, H., Valente, E. G., Gonzalez-Cadavid, N. F. & Rajfer, J. Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculo-protective in the arterial media. *Cardiovasc. Res.* **61**, 796–805 (2004).

59. Ferrini, M. G. *et al.* Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. *Urology* **68**, 429–435 (2006).
60. Ferrini, M. G. *et al.* Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. *Biol. Reprod.* **76**, 915–923 (2007).
61. Kovanecz, I. *et al.* Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int. J. Impot. Res.* **100**, 867–874 (2007).
62. Kovanecz, I. *et al.* Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. *BJU Int.* **101**, 203–210 (2008).
63. Ferrini, M. G. *et al.* Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J. Sex. Med.* **6**, 415–428 (2009).
64. Ferrini, M. G. *et al.* The genetic inactivation of inducible nitric oxide synthase intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. *J. Sex. Med.* (in press).
65. Hochberg, D. *et al.* Interstitial fibrosis of unilateral ureteral obstruction is exacerbated in kidneys of mice lacking the gene for inducible nitric oxide synthase. *Lab. Invest.* **80**, 1721–1728 (2000).
66. Trachtman, H., Futterweit, S., Pine, E., Mann, J. & Valderrama, E. Chronic diabetic nephropathy: role of inducible nitric oxide synthase. *Pediatr. Nephrol.* **17**, 20–29 (2002).
67. Chen, Y. *et al.* Deficiency of inducible nitric oxide synthase exacerbates hepatic fibrosis in mice fed high-fat diet. *Biochem. Biophys. Res. Commun.* **326**, 45–51 (2005).
68. Aram, G., Potter, J. J., Liu, X., Torbenson, M. S. & Mezey, E. Lack of inducible nitric oxide synthase leads to increased hepatic apoptosis and decreased fibrosis in mice after chronic carbon tetrachloride administration. *Hepatology* **47**, 2051–2058 (2008).
69. Bayir, H. *et al.* Enhanced oxidative stress in iNOS deficient mice after traumatic brain injury: support for a neuroprotective role of iNOS. *J. Cereb. Blood Flow Metab.* **25**, 673–684 (2005).
70. Park, K. M. *et al.* Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. *J. Biol. Chem.* **278**, 27256–27266 (2003).
71. Jones, S. P. & Bolli, R. The ubiquitous role of nitric oxide in cardioprotection. *J. Mol. Cell. Cardiol.* **40**, 16–23 (2006).
72. Hesse, M., Cheever, A. W., Jankovic, D. & Wynn, T. A. NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model Of granulomatous disease. *Am. J. Pathol.* **157**, 945–955 (2000).
73. Zhang, P. *et al.* Inducible nitric oxide synthase deficiency protects the heart from systolic overload-induced ventricular hypertrophy and congestive heart failure. *Circ. Res.* **100**, 1089–1098 (2007).
74. Lu, L., Chen, S. S., Hassid, A. & Sun, Y. Cardiac fibrogenesis following infarction in mice with deletion of inducible nitric oxide synthase. *Am. J. Med. Sci.* **335**, 431–438 (2008).
75. Kadhodae, M. *et al.* Proteinuria is reduced by inhibition of inducible nitric oxide synthase in rat renal ischemia-reperfusion injury. *Transplant. Proc.* **41**, 2907–2909 (2009).
76. Kuhlencordt, P. J., Chen, J., Han, F., Astern, J. & Huang, P. L. Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice. *Circulation* **103**, 3099–3104 (2001).
77. Toblli, J. E. *et al.* Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrol. Dial. Transplant.* **24**, 2384–2391 (2009).
78. Mookerjee, I. *et al.* Relaxin inhibits renal myofibroblast differentiation via RXFP1, the nitric oxide pathway, and Smad2. *FASEB J.* **23**, 1219–1229 (2009).
79. Vyas-Read, S., Shaul, P. W., Yuhanna, I. S. & Willis, B. C. Nitric oxide attenuates epithelial-mesenchymal transition in alveolar epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **293**, L212–L221 (2007).
80. Brosius, F. C. 3<sup>rd</sup>. New insights into the mechanisms of fibrosis and sclerosis in diabetic nephropathy. *Rev. Endocr. Metab. Disord.* **9**, 245–254 (2008).
81. Kalk, P. *et al.* Pulmonary fibrosis in L-NAME-treated mice is dependent on an activated endothelin system. *Can. J. Physiol. Pharmacol.* **86**, 541–545 (2008).
82. Peters, H. *et al.* NO mediates antifibrotic actions of L-arginine supplementation following induction of anti-thy1 glomerulonephritis. *Kidney Int.* **64**, 509–518 (2003).
83. Dunkern, T. R., Feurstein, D., Rossi, G. A., Sabatini, F. & Hatzelmann, A. Inhibition of TGF- $\beta$  induced lung fibroblast to myofibroblast conversion by phosphodiesterase inhibiting drugs and activators of soluble guanylyl cyclase. *Eur. J. Pharmacol.* **572**, 12–22 (2007).
84. Masuyama, H. *et al.* Pressure-independent effects of pharmacological stimulation of soluble guanylate cyclase on fibrosis in pressure-overloaded rat heart. *Hypertens. Res.* **32**, 597–603 (2009).
85. Sawada, N. *et al.* Cyclic GMP kinase and RhoA Ser188 phosphorylation integrate pro- and anti-fibrotic signals in blood vessels. *Mol. Cell. Biol.* **29**, 6018–6032 (2009).
86. Knorr, A. *et al.* Nitric oxide-independent activation of soluble guanylate cyclase by BAY 60–2770 in experimental liver fibrosis. *Arzneimittelforschung* **58**, 71–80 (2008).
87. Wang-Rosenke, Y., Neumayer, H. H. & Peters, H. NO signaling through cGMP in renal tissue fibrosis and beyond: key pathway and novel therapeutic target. *Curr. Med. Chem.* **15**, 1396–1406 (2008).
88. Iacono, F. *et al.* Histopathologically proven prevention of post-prostatectomy cavernosal fibrosis with sildenafil. *Urol. Int.* **80**, 249–252 (2008).
89. Vignozzi, L. *et al.* Effect of sildenafil administration on penile hypoxia induced by cavernous neurotomy in the rat. *Int. J. Impot. Res.* **20**, 60–67 (2008).
90. Magheli, A. & Burnett, A. L. Erectile dysfunction following prostatectomy: prevention and treatment. *Nat. Rev. Urol.* **6**, 415–427 (2009).
91. Hennes, A. R., Zaiman, A. & Champion, H. C. PDE 5A inhibition attenuates bleomycin-induced pulmonary fibrosis and pulmonary hypertension through inhibition of ROS generation and RhoA/Rho kinase activation. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **294**, L24–L33 (2008).
92. Wang, Y. *et al.* Enhancing cGMP in experimental progressive renal fibrosis: soluble guanylate cyclase stimulation vs. phosphodiesterase inhibition. *Am. J. Physiol. Renal Physiol.* **290**, F167–F176 (2006).
93. Nagayama, T. *et al.* Sildenafil stops progressive chamber, cellular, and molecular remodeling and improves calcium handling and function in hearts with pre-existing advanced hypertrophy caused by pressure overload. *J. Am. Coll. Cardiol.* **53**, 207–215 (2009).
94. Radovits, T. *et al.* The phosphodiesterase-5 inhibitor vardenafil improves cardiovascular dysfunction in experimental diabetes mellitus. *Br. J. Pharmacol.* **156**, 909–919 (2009).
95. Hohenstein, B., Daniel, C., Wittmann, S. & Hugo, C. PDE-5 inhibition impedes TSP-1 expression, TGF- $\beta$  activation and matrix accumulation in experimental glomerulonephritis. *Nephrol. Dial. Transplant.* **23**, 3427–3436 (2008).
96. Steiropoulos, P., Trakada, G. & Bouros, D. Current pharmacological treatment of pulmonary arterial hypertension. *Curr. Clin. Pharmacol.* **3**, 11–19 (2008).
97. Levine, L. A. & Latchamsetty, K. C. Treatment of erectile dysfunction in patients with Peyronie's disease using sildenafil citrate. *Int. J. Impot. Res.* **14**, 478–482 (2002).
98. Porst, H. *et al.* Long-term safety and efficacy of tadalafil 5 mg dosed once daily in men with erectile dysfunction. *J. Sex. Med.* **5**, 2160–2169 (2008).
99. Bella, A. J., Deyoung, L. X., Al-Numi, M. & Brock, G. B. Daily administration of phosphodiesterase type 5 inhibitors for urological and nonurological indications. *Eur. Urol.* **52**, 990–1005 (2007).

# Acknowledgments

The authors would like to primarily acknowledge the Eli and Edythe Broad Foundation, without whose initial support none of the experimental research work on PD at the UCLA group would have been possible. Additional funding was subsequently applied for some aspects of this research from NIH R01DK-53069, NIH R21DK-070003, Department of Defense PR064756, and NIH G12RR-03026.

The Genetic Inactivation of Inducible Nitric Oxide Synthase (iNOS) Intensifies Fibrosis and Oxidative Stress in the Penile Corpora Cavernosa in Type 1 Diabetes

Monica G. Ferrini, PhD,\*† Steve Rivera, MD,‡ Joanne Moon, undergraduate student,\* Dolores Vernet, PhD,\*‡ Jacob Rajfer, MD,‡§ and Nestor F. Gonzalez-Cadavid, PhD\*‡§  
\*Charles Drew University (CDU), Department of Internal Medicine, Los Angeles, CA, USA; †Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; ‡Los Angeles Biomedical Research Institute (LABioMed) at Harbor-UCLA Medical Center, Division of Urology, Torrance, CA, USA; §David Geffen School of Medicine at UCLA, Department of Urology, Los Angeles, CA, USA

DOI: 10.1111/j.1743-6109.2010.01884.x

ABSTRACT

**Introduction.** Endogenously elicited inducible nitric oxide synthase (iNOS) induction counteracts fibrosis and oxidative stress in penile tissues in rat models of Peyronie’s disease and erectile dysfunction.  
**Aim.** The current study aimed to determine whether the genetic blockade of iNOS expression in the iNOS knock out (iNOS KO) mouse intensifies fibrosis and oxidative stress in the penile corpora cavernosa, and this is exacerbated by streptozotocin (STZ)-induced diabetes and counteracted by insulin.  
**Main Outcomes Measures.** Quantitative assessment of histological and biochemical markers in mouse corporal tissue.  
**Methods.** Male iNOS KO and wild type (WT) mice were left untreated or injected with STZ, with or without insulin treatment. At 8 weeks, glycemia, glucosuria, and proteinuria were determined, and corporal tissue sections were obtained and subjected to Masson trichrome staining for smooth muscle (SM)/collagen ratio, and immunostaining for  $\alpha$ -smooth muscle actin (ASMA) for, SM content, proliferating cell nuclear antigen (PCNA) for cell replication, TGF $\beta$ 1 as profibrotic factor, TUNEL assay for apoptosis, and xanthine oxidoreductase (XOR) for oxidative stress. Collagen was estimated by the hydroxyproline reaction.  
**Results.** The corporal SM/collagen ratio and SM content were reduced, and collagen content increased in iNOS KO mice as compared with WT mice, but apoptosis was decreased and cell replication increased, whereas TGF $\beta$ 1 and XOR did not vary. Severe hyperglycemia caused in the WT a reduction of the corporal SM/collagen ratio and SM content and an increase in apoptosis without changes in PCNA, TGF $\beta$ 1, or XOR. In the iNOS KO mouse the hyperglycemia-induced alterations were exacerbated, with additional increases in oxidative stress and TGF $\beta$ 1. Insulin normalized glycemia and partially protected the SM in both the WT and the iNOS KO mice.  
**Conclusions.** The antifibrotic, antioxidative, and SM-protective roles of iNOS in the penile corpora cavernosa were confirmed in the iNOS KO/STZ mouse model. These findings support the importance of endogenously-elicited iNOS induction in protecting the penile corpora cavernosa from the pro-fibrotic effects of hyperglycemia. **Ferrini MG, Rivera S, Moon J, Vernet D, Rajfer J, and Gonzalez-Cadavid NF. The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. J Sex Med \*\*,\*\*:\*\*-\*\*.**   
**Key Words.** Erectile Dysfunction; Smooth Muscle; Penis; Collagen; Nitric Oxide; Apoptosis

Introduction

Over the last few years it has become increasingly evident in men and in rat models that risk factors of erectile dysfunction (ED) such as

aging, diabetes, hypogonadism, and nerve damage following radical prostatectomy promote smooth muscle (SM) dysfunction in the corpora cavernosa because of the loss or damage of SM, the tissue fibrosis, and the impairment of tissue relaxation



that characterize vasculogenic, more specifically venogenic, ED [1–11]. The association between the increase in collagen fibers and the reduction of SM cells by apoptosis, resulting in the decrease of the SM/collagen ratio, leads to a defective corporal compliance underlying the most prevalent form of ED, corporal veno-occlusive dysfunction (CVD) [12]. Often, but not always, there is a parallel increase in the activation of the TGF $\beta$ 1/Smad key pro-fibrotic pathway and of the production of reactive oxygen species (ROS) that cause oxidative stress and apoptosis and also stimulate fibrosis [7,13,14]. However, TGF $\beta$ 1 expression and oxidative stress in the corpora cavernosa are less marked in CVD than in the tunica albuginea in Peyronie's disease, or in other urological tissues such as in diabetic nephropathy [15–18].

These features in the corpora cavernosa, which are essentially an extension of the vascular system, are accompanied in the case of aging and diabetes by similar changes in the media of both the small penile arteries and large femoral artery and aorta vessels [19,20]. The fibrotic and oxidative stress processes are similar to those seen in Peyronie's disease [15,16], so that in this sense the "hardening" of all these tissues is the common denominator, which in the case of the corporal/vascular system is combined with the SM cell loss, leading to the subsequent functional impairment.

A remarkable constant process in this histopathology is the exacerbated expression of the inducible nitric oxide synthase (iNOS), a NOS isoform that is not expressed in normal penile tissues [21]. Three independent lines of evidence in rodent models support the view that in the penis, iNOS is an endogenous mechanism of defense against fibrosis and SM cell loss and not a deleterious factor contributing to SM dysfunction: (i) gene transfer of iNOS cDNA reduces fibrosis in the corpora and tunica [22,23] in a model of PD; (ii) long-term specific inhibition of iNOS activity by L-NIL intensifies collagen deposition and fibrosis in the penis and arterial wall of old animals [19,24]; and (iii) the sustained pharmacological elevation of the levels of nitric oxide and/or its effector, cyclic guanosine monophosphate, mimicking the result of iNOS induction, also acts as an antifibrotic mechanism in an experimental model of aging and cavernosal nerve resection [1–5,25,26].

This "protective" iNOS induction has also been documented in tissues other than the penis or some arteries using the iNOS knock out mouse (iNOS KO). This strain served to show that in the rat kidney, the anti-inflammatory and antifibrotic

effects of the type-1 cytokine response is iNOS dependent [27] and that iNOS deletion exacerbates interstitial fibrosis after unilateral ureteral obstruction [28], or in streptozotocin (STZ)-induced diabetes despite a compensatory production of endothelial NOS [29]. Similarly hepatic fibrosis in animals fed high-fat diet is exacerbated in the iNOS KO [30].

However, the postulation that iNOS is antifibrotic does not agree with other papers that do not show effects of iNOS deletion on fibrosis, such as in the heart after myocardial infarction [31] and in the liver after chronic carbon tetrachloride administration [32]. There are even reports claiming that it is the iNOS deficiency that provides protection against liver fibrosis in this model [32] and also in overload-induced cardiac hypertrophy [33] and in silica-induced pulmonary fibrosis [34]. It is possible that the deleterious effect of iNOS could be due in some cases to being produced as a result of a macrophage recruitment induced by the chemical insult instead of the progressive and milder metabolic changes caused by diabetes or aging.

Chronic inhibition of iNOS activity in wild type (WT) animals with L-NIL or aminoguanidine partially reduced proteinuria, an indicator of kidney fibrosis in 5/6 nephrectomized rats and ischemia/reperfusion injury [35,36]. In contrast, L-NIL increased myofibroblast accumulation in the "pre-conditioning" of the kidney to protect from ischemia/reperfusion, which agrees with the view that iNOS is essential for protecting the heart from ischemia/reperfusion [37,38]. This confusing evidence regarding the role of iNOS genetic deficiency in fibrosis of nonpenile tissues occurs also in terms of oxidative stress because some reports claim that this ablation is deleterious, and therefore that iNOS counteracts ROS generation, at least in heart and brain injury [39,40], whereas others postulate the opposite, for instance in myocardial infarction and atherosclerotic arteries [41,42].

As no studies are available on the histopathology of penile tissues in the iNOS KO, we decided to approach in this model the clarification of these controversies, focusing on the specific case of the penile corpora cavernosa subjected to the long-term effect of diabetes as a risk factor for ED. We have investigated whether the absence of iNOS in the iNOS KO and/or the development of STZ-induced diabetes neuropathy intensify SM loss, fibrosis, and oxidative stress in the penile corpora cavernosa. The results may help to clarify the role of iNOS in the development of fibrosis due to neuropathies and aging.

## Methods

### Animals and Treatments

All the experiments were approved by the Institutional Animal Care and Use Committee of LABioMed at Harbor-UCLA, Torrance, CA, USA, and according to the NIH Guide for the Care and Use of Laboratory Animals. Two-month old WT C57BL6J mice (WT) and iNOS KO B6.129P2-Nos2tm1Lau/J (iNOS KO) were divided into the following groups that were maintained for 8 weeks before sacrifice (N = 8 mice/group): (i) WT, no treatment control (WT); (ii) WT injected with 150 mg/kg BW STZ to induce diabetes (WT + STZ); (iii) as #2, also injected daily ip with 0.05 IU/Kg BW insulin to exert glycemic control (WT + STZ + INS); (iv) iNOS KO, no treatment control (iNOS KO); (v) iNOS KO injected once ip with 150 mg/kg BW STZ (iNOS KO + STZ); (vi) as #5, also injected ip daily with 0.05 IU/Kg BW insulin (iNOS KO + STZ + INS). Insulin treatment started after the animals showed a glycemia >350 mg/dL.

Body weights were recorded weekly. Blood for biochemical determinations was withdrawn from the tail vein at baseline and then weekly under 3% isoflurane anesthesia. Urine was collected from the urinary bladder under anesthesia before sacrifice. Mice were euthanized by a bolus administration of sodium pentobarbital. The penises were rapidly excised and weighed, the shaft was skin denuded, a mid-region was fixed in 10% formalin for tissue sectioning, and the rest was frozen as fresh tissue at -80°C for other assays.

### Determinations in Fresh Tissue and Blood

Glycemia was determined in serum by an Accu-Chek Active blood glucose meter (Roche, Ireland), and urinary glucose, ketone bodies, specific gravity, pH, and protein were determined by Multistix Dip Stick (Bayer).

4

Collagen estimation in fresh tissue was as previously described [24]. Briefly, the tissue was homogenized in saline, hydrolyzed with 2N NaOH for 30 minutes at 120°C, followed by the measurement of hydroxyproline by a modification of the Neumann and Logan's reaction using Chloramine T and Ehrlich's reagent, against a hydroxyproline standard curve and measuring at 550 nm. Values were expressed as µg of collagen per mg of tissue.

### Histochemistry and Immunohistochemistry

Paraffin embedded tissue sections (5 µm) were used for the following procedures [1-7]: (i) Masson

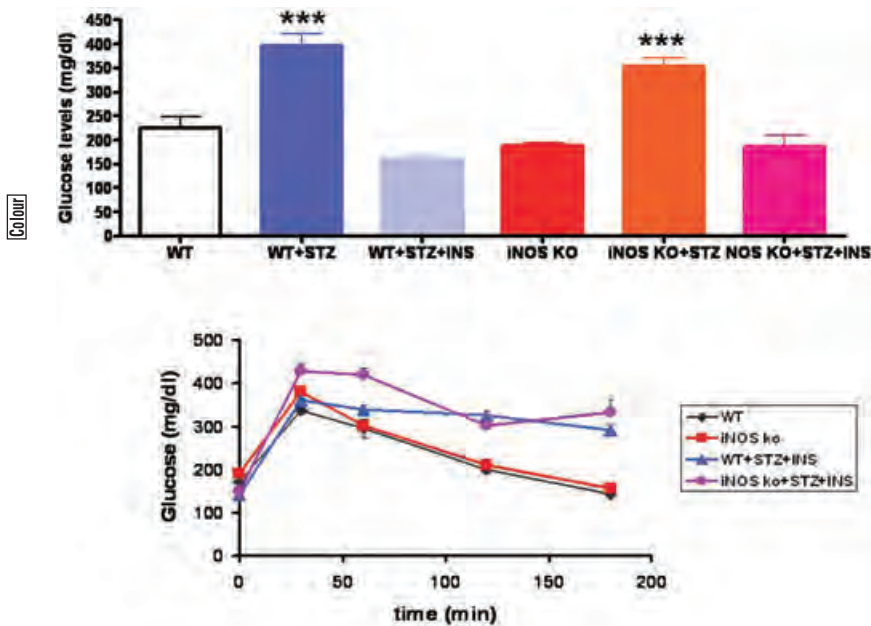
trichrome staining for collagen (blue) and SM cells (red); (ii) immunodetection with (1) monoclonal antibody against α-smooth muscle actin (ASMA) as a SM cell marker (Sigma kit, Sigma Diagnostics, St Louis, MO); (2) polyclonal antibody against TGF-β1 (1:200) (Promega, Madison, WI, USA), as pro-fibrotic factor; (3) monoclonal antibody against proliferating cell nuclear antigen (PCNA) as marker of cell proliferation (1:400) (Chemicon, Temecula, CA, USA); and (iii) polyclonal antibody against xanthine oxidoreductase (XOR) (1:5000; Abcam), as a marker of oxidative stress. The specificity of the antibodies was validated by Western blot.

Briefly, tissue sections were treated with proteinase K (20 µg/mL), followed by quenching in 0.3% H<sub>2</sub>O<sub>2</sub>-PBS, blocked with goat serum (Vector Laboratories, Burlingame, CA, USA) and incubated overnight at 4°C with the primary antibody. In the case of PCNA and XOR, antigen retrieval was performed by boiling the slides for 3 minutes in an antigen unmasking solution (Vector Laboratories). After the overnight incubation with the first antibodies, sections were then incubated with biotinylated anti-Mouse IgG (ASMA, PCNA) or biotinylated anti-Rabbit IgG (TGF-β1, XOR), respectively, followed by ABC complex (Vector Laboratories) and 3,3'-diaminobenzidine (Sigma) (PCNA and iNOS), or with the ASMA Sigma kit (ASMA) and 3-amino-9-ethylcarbazole.

TUNEL assay was performed as described [1-7] by applying the Apoptag peroxidase detection assay (Chemicon), with TdT enzyme and anti-digoxigenin-conjugated peroxidase, and 3,3'-diaminobenzidine/H<sub>2</sub>O<sub>2</sub>. Sections were counter-stained with hematoxylin QS (Vector Laboratories). Negative controls in the immunohistochemical detections were done by replacing the first antibody with IgG isotype. The negative control for TUNEL was by substituting buffer for the TdT enzyme. Testicular tissue sections were used as positive control for TUNEL.

### Quantitative Image Analysis (QIA)

QIA was performed by computerized densitometry using the ImagePro 4.01 program (Media Cybernetics, Silver Spring, MD, USA), coupled to an Leica B microscope equipped with an Spot RT digital camera (Diagnostic Instruments, Portland, OR, USA) [1-7]. For Masson staining, 40× magnification pictures of the whole penis were analyzed for SM cells (stained in red) and collagen (stained in blue) and expressed as SM/collagen ratio. For ASMA and XOR staining, only the



**Figure 1** A single injection of streptozotocin (STZ) at a high dose elicits in the inducible nitric oxide synthase (iNOS) knockout (iNOS KO) mouse a level of hyperglycemia similar to the one in the wild type (WT) mouse, and this is controlled by long-term administration of insulin. Top panel: final values of nonfasting glycemia at 8 weeks after STZ injection, immediately before sacrifice. Bottom panel: oral glucose tolerance test carried out at 7 weeks. WT ± STZ = WT treated with STZ; WT ± STZ ± INS = WT + STZ treated with insulin; iNOS KO ± STZ = iNOS KO treated with STZ; iNOS KO ± STZ ± INS = iNOS KO + STZ treated with insulin. Values are means ± SEM. \*\*\**P* < 0.001, for WT + STZ and iNOS KO + STZ vs. all others.

corpora cavernosa were analyzed in a computerized grid and expressed as % of positive area vs. total area of the corpora cavernosa. For PCNA and TUNEL determinations, the number of positive cells at 400× was counted and results were expressed as a % of positive cells/total cells in the corpora cavernosa. In all cases, four penile anatomically matched tissue sections per animal were examined at 40×, with enough fields to cover the whole corpora cavernosa, and in certain cases at 400× and eight fields per section, with eight animals per group.

Statistical Analysis

Values were expressed as mean ± SEM. The normality distribution of the data was established using the Wilk-Shapiro test, followed by one-way analysis of variance (ANOVA), and post-hoc comparisons with the Bonferroni test, according to the GraphPad Prism V 4.1. The three WT groups were compared among themselves, and the same was done independently for the three iNOS KO groups. When measurements were restricted to two WT and two iNOS KO groups, comparisons were done among all groups. In some instances, the three WT groups were compared among themselves, and the same was done independently for the three iNOS KO groups, as indicated in the figures. For collagen content, comparisons were made only against the control WT by a two-tailed *t*-test. Differences were considered significant at *P* < 0.05.

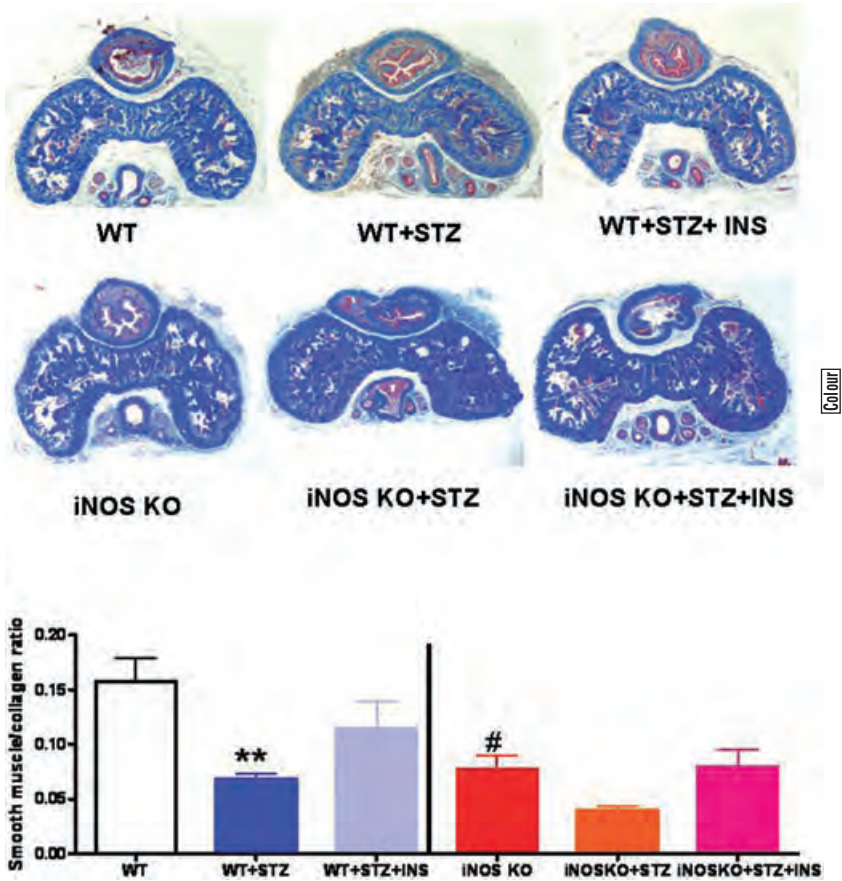
Results

Glycemia was measured weekly in nonfasted animals under isoflurane anesthesia until it raised above a 350 mg/dL threshold in the STZ injected mice, and then immediately before sacrifice. At this point the control WT mice not injected with STZ showed a spontaneous high glycemia (225 ± 23 mg/dL), and to a lower extent this also occurred with the iNOS KO mice (188 ± 5 mg/dL) (Figure 1 top). This appears to be in part inherent to the C57Bl6J genetic background for both strains, where the WT develops a mild hyperglycemia with a regular diet and a marked hyperglycemia with a high-fat diet [43]. But in part it is artifactual too because of nonfasting and the transient effects of isoflurane anesthesia before sacrifice, as confirmed by the fact that when animals were not anesthetized with isoflurane and blood values were obtained after fasting, they were only moderately high in both strains (120–130 mg/dL). Daily insulin treatment normalized glycemia.

In order to corroborate that the nonfasting hyperglycemia in the non-STZ injected animals (control WT and iNOS KO mice) was artifactual, an oral glucose tolerance test in fasting animals was performed 1 week before sacrifice by administering a bolus of glucose and compared with injected the STZ-WT and STZ-iNOS KO animals (diabetic mice). The STZ-injected animals were treated daily with insulin except for the day of the assay, and the animals showed a



**Figure 2** Genetic deletion of inducible nitric oxide synthase (iNOS) causes a reduction in the corporal smooth muscle (SM)/collagen ratio, intensified by diabetes and counteracted by insulin. Top panel: representative micrographies of tissue corporal sections for each group stained with Masson trichrome. Bottom panel: quantitative image analysis for the ratio between areas stained for SM (red) versus collagen (blue). WT = wild type mice; STZ = streptozotocin; WT ± STZ = WT treated with STZ; WT ± STZ ± INS = WT + STZ treated with insulin; iNOS KO = iNOS knockout mice; iNOS KO ± STZ = iNOS KO treated with STZ; iNOS KO ± STZ ± INS = iNOS KO + STZ treated with insulin. Values are means ± SEM. \*\**P* < 0.01; for WT + STZ vs. WT, and iNOS KO+STZ. # *P* < 0.05 for iNOS KO vs. WT.



prolonged hyperglycemia lasting for at least 3 hours while the non-INS- or STZ-injected animals show a normal curve response with a decrease of the glycemic levels after 1 hour, and virtual normalization after 3 hours following the bolus administration (bottom). The latter animals will be referred to as “controls” and considered as nondiabetic.

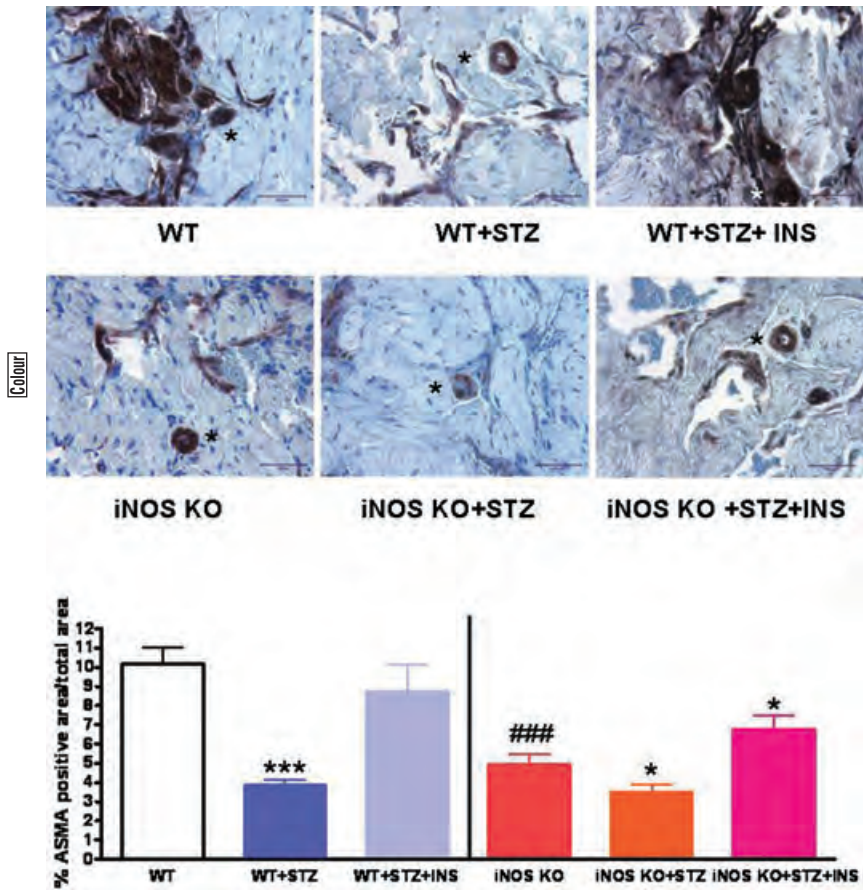
The STZ single injection given to the WT mice virtually doubled the nonfasting glycemia values, making the mice frankly diabetic, and as expected insulin treatment normalized these levels. It took a high dose of STZ for the iNOS KO animals (also given to the controls) to become diabetic, in agreement with the relative resistance of this strain to STZ-induced hyperglycemia reported at lower doses [44]. Proteinuria increased up to eightfold in the STZ-treated animals as compared with controls, but this was completely counteracted by insulin (not shown). No ketonuria was detected in the diabetic animals, and the specific gravity and pH of the urine was similar among all groups. Body weights were reduced by STZ in the WT mice ( $24.0 \pm 1.2$  vs.  $30.3 \pm 0.7$  in the noninjected

controls), and this reduction was partially prevented by insulin, but in the iNOS KO there were no significant changes in body weights by either STZ or STZ and insulin administration (not shown).

Analysis of the corpora cavernosa tissue sections by Masson trichrome staining revealed that there was a considerable reduction of the SM to collagen ratio in the control iNOS KO mice as compared with the control WT mice (Figure 2), as shown by the representative micrographies (top) and QIA (bottom). The hyperglycemia induced by STZ led to a nearly 50% decrease in the WT mice and to a similar but nonsignificant decrease in the iNOS KO mice. Insulin, which had normalized glycemia, also virtually prevented the hyperglycemia-induced reductions in the SM/collagen ratio.

These effects were confirmed by immunodetection of ASMA as a marker of corporal SM cells by both visual inspection (Figure 3 top) and QIA (bottom), except that there was no significant difference upon STZ treatment between the control iNOS KO and the control WT. The decrease in SM cell content in the WT-STZ-treated animals





**Figure 3** Genetic deletion of inducible nitric oxide synthase (iNOS) causes a reduction in the content of corporal smooth muscle (SM), intensified by diabetes and counteracted by insulin. Top panel: representative micrographies of tissue corporal sections for each group immunostained for  $\alpha$ -smooth muscle actin (ASMA). Bottom panel: quantitative image analysis for the ratio between the area stained for SM (brown) vs. the total area. WT = wild type mice; STZ = streptozotocin; WT  $\pm$  STZ = WT treated with streptozotocin STZ; WT  $\pm$  STZ  $\pm$  INS = WT + STZ treated with insulin; iNOS KO = iNOS knockout mice; iNOS KO  $\pm$  STZ = iNOS KO treated with STZ; iNOS KO  $\pm$  STZ  $\pm$  INS = iNOS KO + STZ treated with insulin. Values are means  $\pm$  SEM. \*\*\* $P$  < 0.001; \* $P$  < 0.05 for WT + STZ vs. WT + STZ + INS and WT; and iNOS KO + STZ and iNOS KO + STZ + INS vs. iNOS KO. ### $P$  < 0.001 for iNOS KO vs. WT.

vs. their respective controls was in perfect agreement with the apoptotic index determined by TUNEL (Figure 4). A similar situation occurred with the restoration of SM cell number and the decrease in the apoptotic indexes in the mice treated with insulin. However, the absence of iNOS counteracted the increase of apoptosis by diabetes seen in the WT mice.

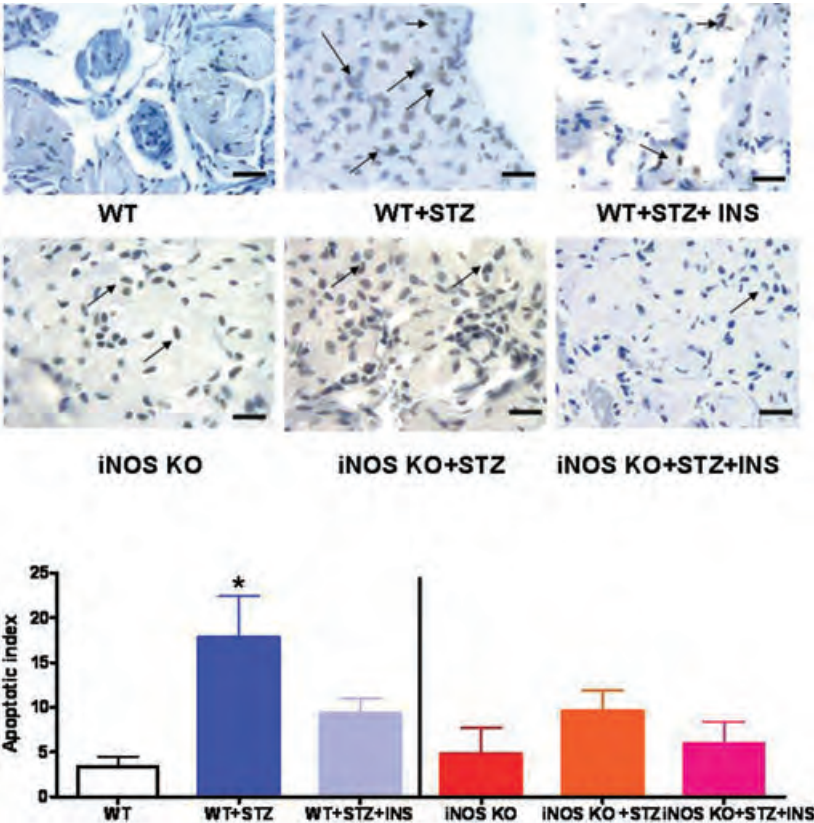
The cell proliferation index determined indirectly by immunostaining for PCNA was doubled in the iNOS KO control as compared with the WT control, but STZ injection did not cause any significant change in any of them (Figure 5 top). As a result, the cell turnover measured by the ratio between proliferation and apoptosis was surprisingly higher in the control iNOS KO vs. the WT (4.0 vs. 1.1) and in the STZ-treated animals (2.8 and 6.4, respectively) vs. the corresponding controls.

As the previous results showed that insulin prevents the histopathological changes seen in the diabetic corpora, no further determinations were considered necessary for the insulin-treated

animals in all the remaining assays. Hydroxyproline determinations in fresh corporal tissue for collagen content expressed in  $\mu\text{g}$  collagen/g fresh tissue showed a significant but modest (14%) increase in the control iNOS KO vs. the control WT ( $4.36 \pm 0.29$  vs.  $3.06 \pm 0.30$ ;  $P$  < 0.05). STZ injection did not change the control value for the iNOS KO ( $4.37 \pm 0.17$ ), and the increase in the value for the WT was marginally nonsignificant ( $4.35 \pm 0.50$ ;  $P$  = 0.06).

The corporal expression of a key pro-fibrotic factor, TGF $\beta$ 1, determined by immunohistochemistry, was not affected by the genetic ablation of iNOS expression, but it was considerably increased by STZ-induced diabetes (Figure 6). Similarly, oxidative stress as denoted by XOR expression in the corporal tissue was marginally higher in the control iNOS KO vs. the WT, but it was the STZ injection in the iNOS KO that induced a remarkable increase, virtually doubling the value (Figure 7). In contrast, as in the case of TGF $\beta$ 1, diabetes in the WT mice was not associated with an increase in oxidative stress.

**Figure 4** Genetic deletion of inducible nitric oxide synthase (iNOS) protects against the apoptosis caused by diabetes in the wild type (WT) mice that is counteracted by insulin. Top panel: representative micrographies of tissue corporal sections for each group immunostained for apoptotic bodies by the TUNEL reaction. Bottom panel: quantitative image analysis for the ratio between the number of apoptotic cells over the total cells counterstained with hematoxylin. STZ = streptozotocin; WT ± STZ = WT treated with STZ; WT ± STZ ± INS = WT + STZ treated with insulin; iNOS KO = iNOS knockout mice; iNOS KO ± STZ = iNOS KO treated with STZ; iNOS KO ± STZ ± INS = iNOS KO + STZ treated with insulin. Values are means ± SEM. \**P* < 0.05 for WT + STZ vs. all others.

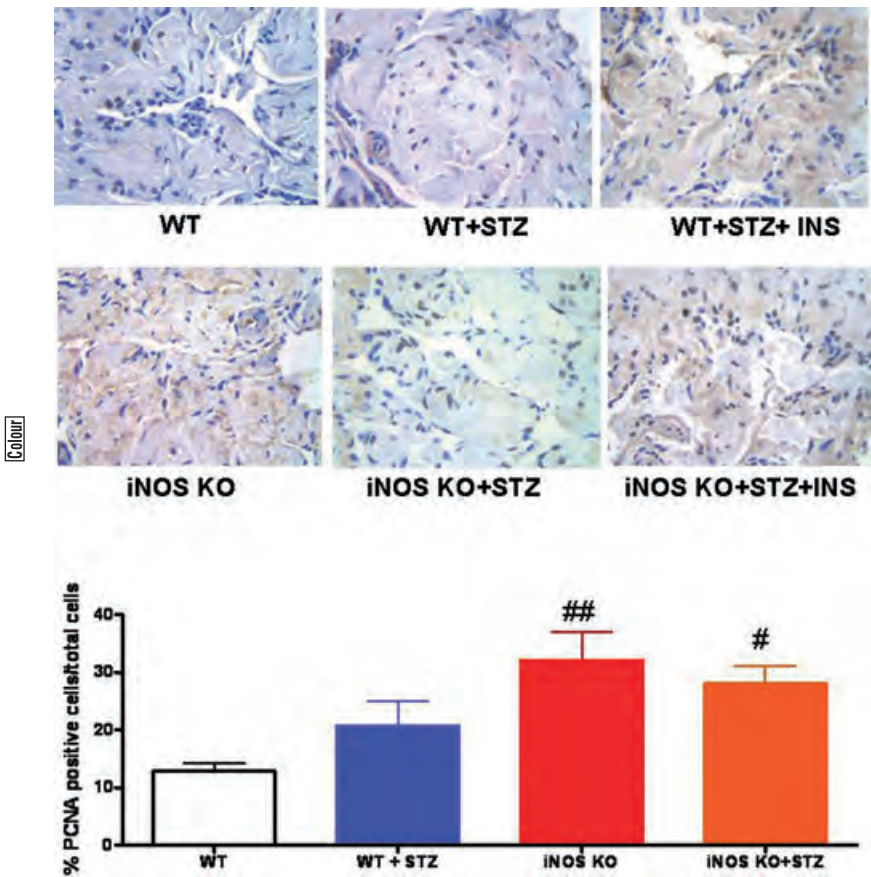


Discussion

This is the first report on the characterization of histopathological and biochemical changes in the corpora cavernosa induced singly by iNOS genetic inactivation or in combination with diabetes. The current results confirm our claims that (i) the loss of SM and the induction of fibrosis and oxidative stress in the corpora are major factors in causing its lack of compliance in diabetes, resulting in ED; and (ii) iNOS spontaneous induction is in this setting a defense mechanism against fibrosis rather than a deleterious factor because the lack of iNOS induced more fibrosis in both the control and diabetic animals. Therefore, in the specific case of the penis of the diabetic iNOS KO, the genetic inactivation of iNOS expression confirms previous results of iNOS inhibition or overexpression in WT rats [3,19,22,23] and provide a more coherent view on the postulated iNOS protective antifibrotic role than the confusing literature in other organs where iNOS is either beneficial in this respect [27–30,37,38] or inactive or noxious [31–36], according to experimental models and conditions. This is not surprising, knowing the biphasic and often antagonistic effects that nitric

oxide exerts in many processes that depend on local tissue concentrations and cell types [45,46], and that are in a way replicated with one of the main pro-fibrotic factors, TGFβ1 [47,48]. We do not believe that the antifibrotic and SM protective effects of iNOS in the penile tissues are due to any fundamental difference with its response in cardiac and renal tissues. First, only the studies involving the genetic deletion or the pharmacological inhibition of iNOS may provide a meaningful answer in this respect, and many of those cited above indicate that iNOS is also protective in myocardial infarction or diabetic nephropathy. Second, leaving aside differences in experimental approaches, such as the experimental condition that elicits the fibrosis and the time frame when it is studied, the most important factors appear to be the intrinsic features of the fibrotic development in each organ and the nature of the key target cells. The drastic and abrupt onset of fibrosis in myocardial infarction, or the progressive and relatively fast development of renal tubulointerstitial fibrosis in type 2 diabetes, differ from the much milder and slower process occurring with aging or diabetes in the penis. As a result, the intensity and duration of





**Figure 5** Genetic deletion of inducible nitric oxide synthase (iNOS) stimulates corporal cell replication, and diabetes does not affect it. Top panel: representative micrographies of tissue corporal sections for each group immunostained for proliferating cells by immunostaining for proliferating cell nuclear antigen (PCNA). Bottom panel: quantitative image analysis for the ratio between the number of proliferating cells over the total cells counterstained with hematoxylin. WT = wild type mice; STZ = streptozotocin; WT ± STZ = WT treated with STZ; iNOS KO = iNOS knockout mice; iNOS KO ± STZ; iNOS KO treated with STZ. Values are means ± SEM. ##*P* < 0.01; #*P* < 0.05 for iNOS KO vs. WT and iNOS KO + STZ vs. WT + STZ.

the inflammatory phase where iNOS is initially produced, and the release of profibrotic cytokines and reactive oxygen species that counteract the beneficial effects of iNOS, are also different, and this may allow in some cases the predominance of iNOS pro-apoptotic effects as previously reported in the brain [49].

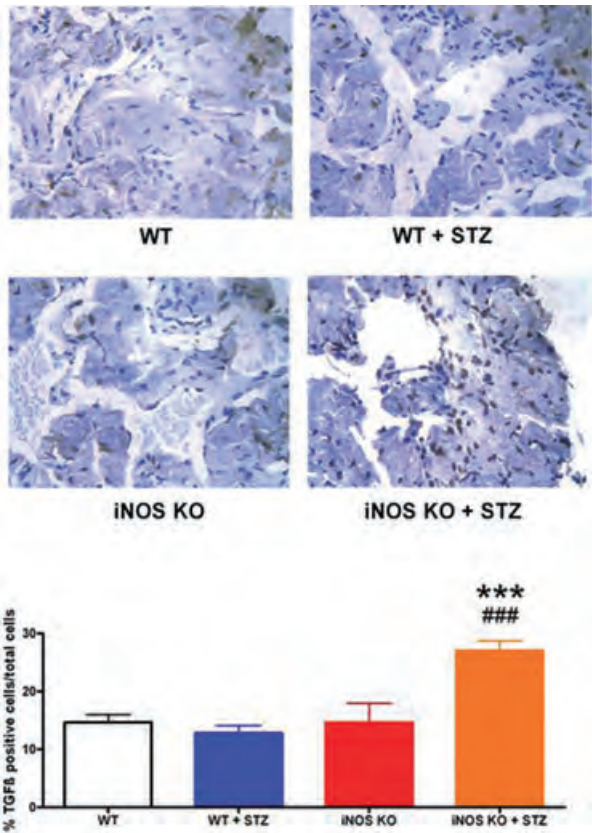
The loss of SM in the corpora and the development of fibrosis and apoptosis in the WT mouse caused by hyperglycemia induced by STZ agree with previous results in rat, mouse, and rabbit models of type 1 diabetes [50–52]. It is logical to assume that these changes are the main cause of the impairment of corporal compliance seen in these models, both in organ bath and *in vivo* in the response to electrical field stimulation [53,54]. In contrast, the relative contribution of corporal endothelial dysfunction, extrapolated from the prevailing views in vascular atherosclerosis research, has not been histologically or mechanistically evaluated conclusively in impotent men or animal models of ED [55–57]. It is surprising that long-term insulin treatment of the WT mouse, which normalized glycemia, was only partially effective to prevent excessive collagen deposition

and apoptosis. This implies that hyperglycemia itself may not be the main inducer of fibrosis and oxidative stress in the corpora cavernosa in this model of type 1 diabetes, or that the intermittent control of glucose due to daily injections was not sufficient to produce a total recovery from the histopathological and biochemical changes induced by diabetes. Corporal damage may also result from a neuropathy of multifactorial origin, and the picture may be even more complicated in models of type 2 diabetes and morbid obesity [6–17].

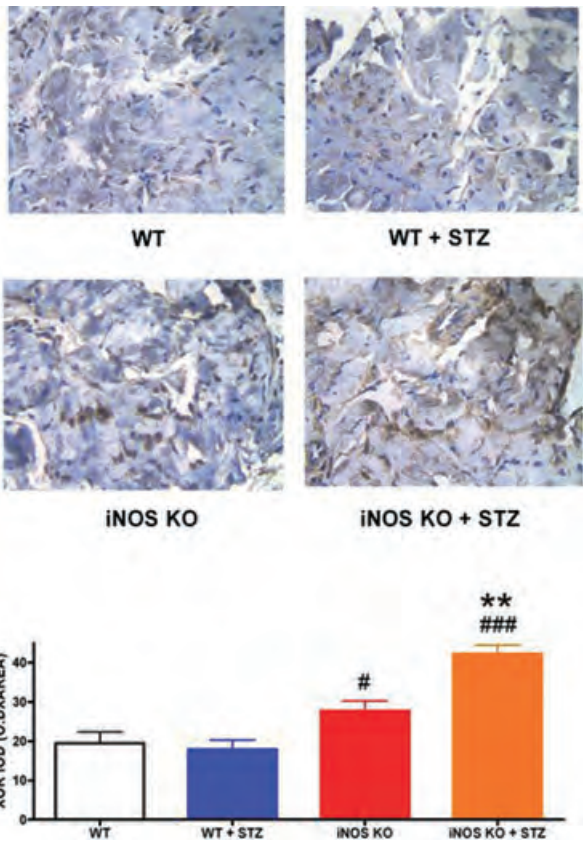
The fact that iNOS deletion per se caused corporal SM loss and fibrosis in nondiabetic animals and intensified them when diabetes was induced by STZ (that also elevated apoptosis and TGFβ1 expression as compared with nondiabetic controls) agrees with our prior results regarding iNOS expression in other models [3,19,23,24] and with some studies in other organs [27–30]. It is noteworthy that oxidative stress measured by XOR levels in corporal tissue was not elevated by diabetes alone in the WT mouse but it was in the iNOS KO, thus reinforcing the assumption that iNOS in this setting may act as an antioxidant by sequester-

ing ROS through the reaction with nitric oxide to form peroxynitrite [21]. Unfortunately, the expression and role of iNOS in the human corpora cavernosa, from the initial description of its induction in human penile SM cells and the cloning of its cDNA [22,58,59], have not been further studied other than for its immunohistochemical detection in some patients with diabetes [60]. However, it has been well characterized in the human Peyronie's disease plaque affecting the tunica albuginea, where its antifibrotic role was first established [24–26], even if this role was not initially acknowledged [61].

In summary, the general antifibrotic and anti-oxidative stress role of iNOS in the penile corpora



**Figure 6** Genetic deletion of inducible nitric oxide synthase (iNOS) causes an increase in corporal TGFβ1 expression only in diabetes. Top panel: representative micrographs of tissue corporal sections for each group immunostained for TGFβ1 (insulin treatments not represented). Bottom panel: quantitative image analysis for the ratio between the number of cells positive for TGFβ1 and the total cells counterstained with hematoxylin. WT = wild type mice; STZ = streptozotocin; WT ± STZ = WT treated with STZ; iNOS KO = iNOS knockout mice; iNOS KO ± STZ = iNOS KO treated with STZ. Values are means ± SEM. \*\*\**P* < 0.001 for iNOS KO + STZ vs. iNOS KO. ###*P* < 0.001 for iNOS KO + STZ vs. WT + STZ.



**Figure 7** Genetic deletion of inducible nitric oxide synthase (iNOS) does not affect corporal oxidative stress, but diabetes does induce it. Top panel: representative micrographs of tissue corporal sections for each group immunostained for oxidative stress by immunostaining for xanthine oxidoreductase (XOR). Bottom panel: quantitative image analysis for the ratio between the positive vs. total areas expressed in integrated optical density (IOD). WT = wild type mice; STZ = streptozotocin; WT ± STZ = WT treated with STZ; iNOS KO = iNOS knockout mice; iNOS KO ± STZ = iNOS KO treated with STZ. Values are means ± SEM. \*\**P* < 0.01 for iNOS KO + STZ vs. iNOS KO. ###*P* < 0.001; #*P* < 0.05 for iNOS KO vs. WT and iNOS KO + STZ vs. WT + STZ.

cavernosa previously shown for aging and cavernosal nerve damage was confirmed and extended to type I diabetic animals by showing that the inactivation of the iNOS gene exacerbates corporal fibrosis in diabetes.

**Acknowledgements**

Supported by Award Numbers 07-05RA-44 (American Diabetes Association); NIH 5RO1DK53069 (NGC) and partially by SC1NS064611 from NINDS and NIGMS (MGF).

**Corresponding Author:** Nestor F. Gonzalez-Cadavid, PhD, Division of Urology, Los Angeles Biomedical



Research Institute (LABioMed) at Harbor-UCLA Medical Center, 1000 West Carson St Torrance California United States 90509, 1000 West Carson St, Los Angeles, CA 90509, USA. Tel: ••; Fax: ••; E-mail: ncadavid@ucla.edu

*Conflict of Interest:* None.

## Statement of Authorship

### Category 1

#### (a) Conception and Design

Nestor F. Gonzalez-Cadavid

#### (b) Acquisition of Data

Monica G Ferrini; Steve Rivera; Joanne Moon; Dolores Vernet

#### (c) Analysis and Interpretation of Data

Nestor F. Gonzalez-Cadavid; Monica G. Ferrini; Steve Rivera; Dolores Vernet

### Category 2

#### (a) Drafting the Article

Nestor F. Gonzalez-Cadavid; Monica G. Ferrini; Jacob Rajfer

#### (b) Revising It for Intellectual Content

Nestor F. Gonzalez-Cadavid; Monica G. Ferrini; Jacob Rajfer

### Category 3

#### (a) Final Approval of the Completed Article

Monica G. Ferrini; Steve Rivera; Joanne Moon; Dolores Vernet; Jacob Rajfer; Nestor F. Gonzalez-Cadavid

## References

- Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Med* 2009;6:415–28.
- Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. *BJU Int* 2008;101:203–10.
- Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res* 2007;100:867–74.
- Ferrini MG, Davila H, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF, Rajfer J. Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. *Urology* 2006;68:429–35.
- Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. *Biol Reprod* 2007;76:915–23.
- Kovanecz I, Ferrini MG, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. *BJU Int* 2007;100:867–74.
- Kovanecz I, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int* 2006;98:116–24.
- Iacono F, Giannella R, Somma P, Manno G, Fusco F, Mirone V. Histological alterations in cavernous tissue after radical prostatectomy. *J Urol* 2005;173:1673–86.
- McCullough A. Penile change following radical prostatectomy: Size, smooth muscle atrophy, and curve. *Curr Urol Rep* 2008;9:492–9.
- Schwartz EJ, Wong P, Graydon RJ. Sildenafil preserves intracorporeal smooth muscle after radical retropubic prostatectomy. *J Urol* 2004;171:771–4.
- Blute M, Hakimian P, Kashanian J, Shteynshlyuger A, Lee M, Shabsigh R. Erectile dysfunction and testosterone deficiency. *Front Horm Res* 2009;37:108–22.
- Tal R, Valenzuela R, Aviv N, Parker M, Waters WB, Flanagan RC, Mulhall JP. Persistent erectile dysfunction following radical prostatectomy: The association between nerve-sparing status and the prevalence and chronology of venous leak. *J Sex Med* 2009;6:2813–9.
- Zhang LW, Piao S, Choi MJ, Shin HY, Jin HR, Kim WJ, Song SU, Han JY, Park SH, Mamura M, Kim SJ, Ryu JK, Suh JK. Role of increased penile expression of transforming growth factor-beta1 and activation of the Smad signaling pathway in erectile dysfunction in streptozotocin-induced diabetic rats. *J Sex Med* 2008;5:2318–29.
- Ryu JK, Song SU, Choi HK, Seong DH, Yoon SM, Kim SJ, Suh JK. Plasma transforming growth factor-beta1 levels in patients with erectile dysfunction. *Asian J Androl* 2004;6:349–53.
- Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF. Pro-fibrotic role of myostatin in Peyronie's disease. *J Sex Med* 2008;5:1607–22.
- Gonzalez-Cadavid NF, Rajfer J. Experimental models for Peyronie's disease. Implications for therapy. *J Sex Med* 2009;6:303–13.
- Toblli JE, Ferrini MG, Cao G, Vernet D, Angerosa M, Gonzalez-Cadavid NF. Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrol Dial Transpl* 2009;24:2384–91.
- Sarafidis PA, Bakris GL. Protection of the kidney by thiazolidinediones: An assessment from bench to bedside. *Kidney Int* 2006;70:1223–33.
- Ferrini MG, Davila H, Valente EG. (Gonzalez-Cadavid NF\*, Rajfer J\*, \*equal contributors). Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculo-protective in the arterial media. *Cardiovascular Res* 2007;61:796–805.
- Kovanecz I, Nolzco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. *BJU Int* 2009;103:1396–404.
- Gonzalez-Cadavid NF, Rajfer J. The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. *Curr Pharm Des* 2005;11:4041–6.
- Garban H, Marquez D, Magee T, Moody J, Rajavashist T, Rodriguez JA, Hung A, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Cloning of rat and human inducible penile nitric oxide synthase. Application for gene therapy of erectile dysfunction. *Biol Reprod* 1997;56:954–63.
- Davila HH, Magee TR, Rajfer J, Gonzalez-Cadavid NF. Gene therapy with the inducible nitric oxide synthase (iNOS) cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease. *Biol Reprod* 2004;71:1568–77.

- 24 Ferrini MG, Vernet D, Magee TR, Shahed A, Quian A, Rajfer J, Gonzalez-Cadavid NF. Antifibrotic role of inducible nitric oxide synthase (iNOS). *Nitric Oxide* 2002;6:283–94.
- 25 Vernet D, Ferrini MG, Valente E, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF. Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. *Nitric Oxide* 2002;7:262–76.
- 26 Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, Gonzalez-Cadavid NF. L-arginine and PDE inhibitors counteract fibrosis in Peyronie's plaque and related fibroblast cultures. *Nitric Oxide* 2003;9:229–44.
- 27 Hesse M, Cheever AW, Jankovic D, Wynn TA. NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *Am J Pathol* 2000;157:945–55.
- 28 Hochberg D, Johnson CW, Chen J, Cohen D, Stern J, Vaughan ED Jr, Poppas D, Felsen D. Interstitial fibrosis of unilateral ureteral obstruction is exacerbated in kidneys of mice lacking the gene for inducible nitric oxide synthase. *Lab Invest* 2000;80:1721–8.
- 29 Trachtman H, Futterweit S, Pine E, Mann J, Valderrama E. Chronic diabetic nephropathy: Role of inducible nitric oxide synthase. *Pediatr Nephrol* 2002;17:20–9.
- 30 Chen Y, Hozawa S, Sawamura S, Sato S, Fukuyama N, Tsuji C, Mine T, Okada Y, Tanino R, Ogushi Y, Nakazawa H. Deficiency of inducible nitric oxide synthase exacerbates hepatic fibrosis in mice fed high-fat diet. *Biochem Biophys Res Commun* 2005;326:45–51.
- 31 Lu L, Chen SS, Hassid A, Sun Y. Cardiac fibrogenesis following infarction in mice with deletion of inducible nitric oxide synthase. *Am J Med Sci* 2008;335:431–8.
- 32 Aram G, Potter JJ, Liu X, Torbenson MS, Mezey E. Lack of inducible nitric oxide synthase leads to increased hepatic apoptosis and decreased fibrosis in mice after chronic carbon tetrachloride administration. *Hepatology* 2008;47:2051–8.
- 33 Zhang P, Xu X, Hu X, van Deel ED, Zhu G, Chen Y. Inducible nitric oxide synthase deficiency protects the heart from systolic overload-induced ventricular hypertrophy and congestive heart failure. *Circ Res* 2007;100:1089–98.
- 34 Zeidler P, Hubbs A, Battelli L, Castranova V. Role of inducible nitric oxide synthase-derived nitric oxide in silica-induced pulmonary inflammation and fibrosis. *J Toxicol Environ Health A* 2004;67:1001–26.
- 35 Bautista-García P, Sánchez-Lozada LG, Cristóbal-García M, Tapia E, Soto V, Avila-Casado MC, Márquez-Velasco R, Bojalil R, Franco M, Herrera-Acosta J. Chronic inhibition of NOS-2 ameliorates renal injury, as well as COX-2 and TGF-beta 1 overexpression in 5/6 nephrectomized rats. *Nephrol Dial Transplant* 2006;21:3074–81.
- 36 Kadkhodae M, Zahmatkesh M, Sadeghipour HR, Eslamifar A, Taeb J, Shams A, Mahdavi-Mazdeh M. Proteinuria is reduced by inhibition of inducible nitric oxide synthase in rat renal ischemia-reperfusion injury. *Transplant Proc* 2009;41:2907–9.
- 37 Park KM, Byun JY, Kramers C, Kim JI, Huang PL, Bonventre JV. Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. *J Biol Chem* 2003;278:27256–66.
- 38 Jones SP, Bolli R. The ubiquitous role of nitric oxide in cardioprotection. *J Mol Cell Cardiol* 2006;40:16–23.
- 39 Bayir H, Kagan VE, Borisenko GG, Tyurina YY, Janesko KL, Vagni VA, Billiar TR, Williams DL, Kochanek PM. Enhanced oxidative stress in iNOS deficient mice after traumatic brain injury: Support for a neuroprotective role of iNOS. *J Cereb Blood Flow Metab* 2005;25:673–84.
- 40 Imamura G, Bertelli AA, Bertelli A, Otani H, Maulik N, Das DK. Pharmacological preconditioning with resveratrol: An insight with iNOS knockout mice. *Am J Physiol Heart Circ Physiol* 2002;282:H1996–2003.
- 41 Liu YH, Carretero OA, Cingolani OH, Liao TD, Sun Y, Xu J, Li LY, Pagano PJ, Yang JJ, Yang XP. Role of inducible nitric oxide synthase in cardiac function and remodeling in mice with heart failure due to myocardial infarction. *Am J Physiol Heart Circ Physiol* 2005;289:H2616–23.
- 42 Kuhlencordt PJ, Chen J, Han F, Astern J, Huang PL. Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice. *Circulation* 2001;103:3099–104.
- 43 Agardh CD, Agardh E, Hultberg B, Åhrén B. Long-standing hyperglycemia in C57BL/6J mice does not affect retinal glutathione levels or endothelial/pericyte ratio in retinal capillaries. *J Diabetes Complications* 2000;14:146–53.
- 44 Flodström M, Tyrberg B, Eizirik DL, Sandler S. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to multiple low-dose streptozotocin-induced diabetes. *Diabetes* 1999;48:706–13.
- 45 Lu XM, Zhang GX, Yu YQ, Kimura S, Nishiyama A, Matsuyoshi H, Shimizu J, Takaki M. The opposite roles of nNOS in cardiac ischemia-reperfusion-induced injury and in ischemia pre-conditioning-induced cardioprotection in mice. *J Physiol Sci* 2009;59:253–62.
- 46 Ridnour LA, Thomas DD, Donzelli S, Espey MG, Roberts DD, Wink DA, Isenberg JS. The biphasic nature of nitric oxide responses in tumor biology. *Antioxid Redox Signal* 2006;8:1329–37.
- 47 Bachman KE, Park BH. Dual nature of TGF-beta signaling: Tumor suppressor vs. tumor promoter. *Curr Opin Oncol* 2005;17:49–54.
- 48 Ashcroft GS. Bidirectional regulation of macrophage function by TGF-beta. *Microbes Infect* 1999;1:1275–82.
- 49 Ferrini M, Wang C, Swerdloff RS, Sinha Hikim AP, Rajfer J, Gonzalez-Cadavid NF. Aging-related increased expression of inducible nitric oxide synthase and cytotoxicity markers in rat hypothalamic regions associated with male reproductive function. *Neuroendocrinology* 2001;74:1–11.
- 50 Park K, Ryu KS, Li WJ, Kim SW, Paick JS. Chronic treatment with a type 5 phosphodiesterase inhibitor suppresses apoptosis of corporal smooth muscle by potentiating Akt signalling in a rat model of diabetic erectile dysfunction. *Eur Urol* 2008;53:1282–8.
- 51 Xie D, Odronic SI, Wu F, Phippen A, Donatucci CF, Annex BH. Mouse model of erectile dysfunction due to diet-induced diabetes mellitus. *Urology* 2007;70:196–201.
- 52 Bozkurt NB, Pekiner C. Impairment of endothelium- and nerve-mediated relaxation responses in the cavernosal smooth muscle of experimentally diabetic rabbits: Role of weight loss and duration of diabetes. *Naunyn Schmiedebergs Arch Pharmacol* 2006;373:71–8.
- 53 Cartledge JJ, Eardley I, Morrison JF. Nitric oxide-mediated corpus cavernosal smooth muscle relaxation is impaired in ageing and diabetes. *BJU Int* 2001;87:394–401.
- 54 Büyükaşar K, Un I. Effects of the Rho-kinase inhibitors, Y-27632 and fasudil, on the corpus cavernosum from diabetic mice. *Eur J Pharmacol* 2003;472:235–8.
- 55 Aversa A, Greco E, Bruzziches R, Pili M, Rosano G, Spera G. Relationship between chronic tadalafil administration and improvement of endothelial function in men with erectile dysfunction: A pilot study. *Int J Impot Res* 2007;19:200–7.
- 56 Musicki B, Burnett AL. Endothelial dysfunction in diabetic erectile dysfunction. *Int J Impot Res* 2007;19:129–38.
- 57 Jones RW, Jeremy JY, Koupparis A, Persad R, Shukla N. Cavernosal dysfunction in a rabbit model of hyperhomocysteinemia. *BJU Int* 2005;95:125–30.
- 58 Hung A, Vernet D, Xie Y, Rajavashisth T, Rodríguez JA, Rajfer J, González-Cadavid NF. Expression of inducible nitric

1	oxide synthase in smooth muscle cells from rat penile corpora	Advanced glycation end products in human penis: Elevation in	9
2	cavernosa. J Androl 1995;16:469–81.	diabetic tissue, site of deposition, and possible effect through	10
3	59 Rajasekaran M, Mondal D, Agrawal K, Chen IL, Hellstrom	iNOS or eNOS. Urology 1997;50:1016–26.	11
4	W, Sikka S. Ex vivo expression of nitric oxide synthase iso-	61 Bivalacqua TJ, Diner EK, Novak TE, Vohra Y, Sikka SC,	12
5	forms (eNOS/iNOS) and calmodulin in human penile caver-	Champion HC, Kadowitz PJ, Hellstrom WJ. A rat model of	13
6	nosal cells. J Urol 1998;160:2210–5.	Peyronie’s disease associated with a decrease in erectile activity	14
7	60 Seftel AD, Vaziri ND, Ni Z, Razmjouei K, Fogarty J, Hampel	and an increase in inducible nitric oxide synthase protein	15
8	N, Polak J, Wang RZ, Ferguson K, Block C, Haas C.	expression. J Urol 2000;163:1992–8.	16

**BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME <b>GONZALEZ-CADAVID, NESTOR F</b>	POSITION TITLE <b>Professor</b>		
eRA COMMONS USER NAME (credential, e.g., agency login) <b>NESTORGON</b>			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
University of Buenos Aires, Argentina	M.Sc.	1961	Biochemistry
University of Buenos Aires, Argentina	Ph.D.	1964	Biochemistry
University of London, England	Ph.D.	1967	Biochemistry

**A. Personal Statement**

The goal of this project is to demonstrate that the Oct-4 Pr-gfp transgenic mouse turned diabetic by streptozotocin injection is an excellent model to study: a) the effects of diabetes on the proliferation and differentiation of Oct-4 expressing penile stem cells (**OPSC**), and to define the impact on their gene expression profile; and b) the counteraction by implanted MDSC in the corpora of the progression of diabetes-related corporal histopathology and the resulting erectile dysfunction, in part by the stimulation of the number and differentiation of OPSC. We speculate that the diabetic Oct-4 Pr-gfp mouse may be used not just for this purpose but for similar approaches to characterize the impact of diabetes on exogenous/endogenous stem cells interactions in a variety of organs and conditions.

I believe that I am well qualified to act as PI based on the following facts: a) being a full professor, DOD/NIH funded investigator, and senior author in most of our papers, and obviously, having written this project; b) having extensive expertise on translational molecular and cellular biology, focused in part in the last 17 years on erectile dysfunction, evolving in the last decade to the study of basic mechanisms of fibrosis in the penis underlying this process, and also in other urogenital and skeletal muscle disease in both rodent models and cell cultures; c) more recently studying the modulation of multipotent cell line differentiation and specifically the use of muscle derived stem cells (MDSC) for tissue repair and to combat fibrosis in several organs, including the penis; c) demonstrating the histopathological and functional effects of types 1 and 2 diabetes on the penis and other organs in rodent models; and d) having isolated and characterized putative endogenous stem cells from the penis and kidney using the Oct-4 Pr-gfp mouse

**B. Positions and Honors.**

1961 Gold medal to the best MSc graduate, University of Buenos Aires  
 1961-62 Fellowship, National Council of Scientific Research (Argentina)  
 1964 Gold Medal to the best doctorate dissertation, University of Buenos Aires  
 1964-66 Fellowship, Natl Council Sci Res (Argentina), work at the Courtauld Inst Biochemistry, London Univ.  
 1967 Fellowship, WellcomeTrust (England), *ibid*  
 1968-71 Assoc. Professor, Dept. Biochemistry., Sch. of Science, Central University, Caracas, Venezuela  
 1971-92 Full Professor, Dept Cell Biology, School of Science, Central University, Caracas, Venezuela  
 1978-79 Gosney Visit. Assoc. in Biology, California Institute of Technology, Biology Division, Pasadena, CA  
 1980 Senior Fellowship, Guggenheim Foundation, Cal. Inst. of Technology, Biology Div., Pasadena, CA  
 1982 Visiting Professor, University of Buenos Aires, School of Biochemistry, Buenos Aires, Argentina  
 1984 Fellowship, Internatl Union Against Cancer, City of Hope Med Center, Div Biology, Duarte (CA).  
 1987-88 Visiting Professor, UCLA School of Medicine, Div of Hematology/Oncology, Los Angeles, CA  
 1987 E. Roosevelt fellowship, Internatl Union Against Cancer, UCLA Med School, Dept Medicine, Los Angeles (CA); Senior Fellowship, United Nations Univ. *ibid*



1990-92 National Research Service Award (Senior Fellowship), Popul. Res. Center, Harbor/UCLA Med. Ctr.  
1990-96 Adj. Associate Professor, Dept of Surgery/Urology, UCLA School of Medicine, Director Urology Research Laboratory, Harbor-UCLA REI  
1996-on Adjunct Professor, Department of Urology, UCLA School of Medicine, and Director, as above  
1997-on Professor, Dept of Internal Medicine/Endocrinology, Charles R. Drew University.  
2001-07 Director, RCMI Molecular Medicine Core, Charles R. Drew University  
2002-08 Associate Director, Androgen Center, Charles R. Drew University  
2009-13 Member, NIH UKGD Urology Study Section  
2010-15 Editor-in-Chief, International Journal of Impotence Research, Nature Publishing Corp.

### **C. Selected peer-reviewed publications**

From a list of 165 on CV. For Pubmed search, please use Gonzalez-Cadavid N, or the last name only, and not NF, to avoid missing 40 odd papers

#### **Most relevant (multipotent/stem cells; diabetes rodent models; erectile dysfunction)**

1. Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Fareez MM, **Gonzalez-Cadavid NF** (2005) Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. *Endocrinology* 146:3547-3557. PMID: 15878958
2. Vernet D, Nolzco G, Cantini L, Magee TR, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. *Biol Reprod*, 73:1199-1210. PMID: 16093362
3. Ferrini MG, Nolzco G, Vernet D, **Gonzalez-Cadavid NF\***, Berman J\* (\*: equal contributors) (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. *Fertil Steril*, 86:1152-1163. PMID: 16978624
4. Kovanecz I, Ferrini MG, Vernet D, Nolzco G, Rajfer J, **Gonzalez-Cadavid NF** (2006). Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int*, 98:116-124. PMID: 16831155
5. Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, **Gonzalez-Cadavid NF** (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med*, 4(3):633-43.
6. Kovanecz I, Ferrini MG, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. *BJU Int*. 2007 Oct; 100(4):867-74.
7. Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid NF** (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. *BJU Int* 101(2):203-10.
8. Nolzco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, **Gonzalez-Cadavid NF** (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int*, 101(9):1156-64. PMID: 18294308
9. Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, **Gonzalez-Cadavid NF** (2008) Myostatin promotes a fibrotic phenotypic switch in multipotent C3h 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol* 196:235-49. PMID: 18252947
10. Kovanecz I, Nolzco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF** (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. *BJU Int*, 2009, 103(10):1396-404. PMID: 19154511
11. Toblli JE; Ferrini MG; Cao G; Vernet D; Angerosa M; **Gonzalez-Cadavid NF** (2009) Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrol Dial Transpl*, Aug;24(8):2384-91. PMID: 19297362
12. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, **Gonzalez-Cadavid NF** (2009). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Med*, 6(2):415-28
13. **Gonzalez-Cadavid NF** (2009) Mechanisms of penile fibrosis. *J Sex Med*, 6 Suppl 3:353-62.
14. Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini MG, Bhatia NN, **Gonzalez-Cadavid NF** (2009) Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds. *Obst Gynecol*, Aug;114(2, Part 1):300-309 PMID: 19622991

15. Toblli JE, Cao G, Angerosa M, **Gonzalez-Cadavid NF** (2010) The antifibrotic effects of pioglitazone at low doses on the diabetic rat kidney are associated with the improvement of markers of cell turnover, angiogenesis and endothelial dysfunction. *Kidney Blood Press Res*, preliminary acceptance.
16. Ferrini MG, Moon J, Rivera S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF** (2010) The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. *J Sex Med*, in press

#### **Other (2005-2010, including erectile dysfunction)**

- Davila H, Magee TR, Rajfer J, **Gonzalez-Cadavid NF** (2005). Peyronie's disease is associated with an increase of plasminogen activator inhibitor-1 (PAI-1) at the RNA and protein levels. *Urology*, 65:645-648
- Hikim AP, Vera Y, Vernet D, Castaneres M, Diaz-Romero M, Ferrini M, Swerdloff RS, **Gonzalez-Cadavid NF**, Wang C (2005) Involvement of nitric oxide-mediated intrinsic pathway signaling in age-related increase in germ cell apoptosis in male brown-norway rats. *J Gerontol A Biol Sci Med Sci* 60:702-708.
5. Jasuja R, Ramaraj P, Mac RP, Singh AB, Storer TW, Artaza J, Miller A, Singh R, Taylor WE, Lee ML, Davidson T, Sinha-Hikim I, **Gonzalez-Cadavid N**, Bhasin S (2005) Delta-4-androstene-3,17-dione binds androgen receptor, promotes myogenesis in vitro, and increases serum testosterone levels, fat-free mass, and muscle strength in hypogonadal men. *J Clin Endocrinol Metab*. 90:855-863. PMID: 15522925
- Gonzalez-Cadavid NF**, Rajfer J (2005) The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. *Curr Pharm Des* 11:4041-4046
- Gonzalez-Cadavid NF**, Rajfer J (2005) Mechanisms of disease: new insights into the cellular and molecular pathology of Peyronie's disease. *Nature Clin Pract Urol*, 2:291-298.
- Saenz de Tejada I, Angulo J, Celtek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Physiology of erectile function. *J Sex Med* 1:254-265.
- Saenz de Tejada I, Angulo J, Celtek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Pathophysiology of erectile dysfunction. *J Sex Med* 2:26-39.
- Ferrini MG, Kovanecz I, Nolasco G, Rajfer J, **Gonzalez-Cadavid NF** (2006) Effects of long-term treatment with vardenafil on the development of the fibrotic plaque in a rat model of Peyronie's disease *BJU Int*, 97:625-633
- Vernet D, Magee TR, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2006) Long-term continuous incubation with high doses of tadalafil does not up-regulate the levels of phosphodiesterase 5 (pde5) in cultures of human penile smooth muscle cells. *J Sex Med* 3:84-94; discussion 94-95
7. Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, **Gonzalez-Cadavid NF**, Bhasin S (2006) Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinology*. 2006 Jan;147(1):141-54. PMID: 16210377
- Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, **Gonzalez-Cadavid NF** (2006). Myostatin shRNA gene therapy increases muscle mass. *J Gene Med* 8:1171-81
- Ferrini MG, Davila H, Kovanecz I, Sanchez S, **Gonzalez-Cadavid NF\*** Rajfer J\* (\*: equal contributors) (2006) Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. *Urology*, 2006 Aug;68(2):429-35.
- Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. *Biol Reprod*, 76:915-923
- Artaza JN, Reisz-Porszasz S, Dow JS, Kloner RA, Tsao J, Bhasin S, **Gonzalez-Cadavid NF** (2007) Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. *J Endocrinol*, 194(1):63-76.
- Gonzalez-Cadavid NF**, Rajfer J (2007) Experimental models for the study of the cellular and molecular pathophysiology of Peyronie's disease. In: *Current Clinical Urology: Peyronie's Disease, A Guide to Clinical Management*, ed by L.A. Levine, Humana Press, Totowa, NJ, p 19-39
- Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVD) induced by cavernosal nerve resection in rats. *Int J Impot Res*, 2007, 100(4):867-74.
- Rambhatla A, Kovanecz I, Ferrini M, **Gonzalez-Cadavid NF**, Rajfer J (2008) Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. *Int J Impot Res*. 20(1):30-4. Cantini

LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, **Gonzalez-Cadavid NF** (2008) Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med, 5(7):1607-22

Braga M, Sinha Hikim AP, Datta S, Ferrini MG, Brown D, Kovacheva EL, **Gonzalez-Cadavid NF**, Sinha-Hikim I (2008) Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. Apoptosis. 2008 Jun;13(6):822-32.

**Gonzalez-Cadavid NF**, Rajfer J (2009) Experimental models for Peyronie's disease. Implications for therapy. J Sex Medic, 6(2):303-13

Tsao J, Vernet D, Gelfand R, Kovanecz I, Nolzco G, **Gonzalez-Cadavid NF** (2010) The genetic inactivation of myostatin or dystrophin expression inhibits the myogenic differentiation of a population of stem cells from the mouse skeletal muscle. J Endocr, submitted

**Gonzalez-Cadavid NF**, Rajfer J (2010) Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. Nat Rev Urol.7(4):215-21.

## D. Research Support

### Funded, ongoing

1. PR064756 (PI: Gonzalez-Cadavid) Department of Defense 03/01/07-02/28/10 (no cost extension to 02/11)  
Pharmacological prevention and reversion of erectile dysfunction after radical prostatectomy, by modulation of nitric oxide/cGMP pathways
2. PC061300 (PI: Gonzalez-Cadavid) Department of Defense 03/31/07-02/28/11  
Modulation of stem cell differentiation and myostatin as an approach to counteract fibrosis in dystrophic muscle regeneration after injury.
3. U54 CA14393-01. NCI Pilot 2 PI: Gonzalez-Cadavid; Program Director: Vadgama) 01/01/10-08/31/12  
Potential oncogenic effects of alcohol on breast stem cells (previous: Mechanism of alcohol-associated breast cancer)

### Pending

1. NIH R21ES019465-01 (PI: Gonzalez-Cadavid) 06/01/10-05/31/12  
Bisphenol A effects on the peripheral mechanisms of penile erection
2. NIH 1R21DK089996-01 (PI: Gonzalez-Cadavid) 07/01/10-06/30/12  
Human iPS in erectile dysfunction after radical prostatectomy in rat models

### Recent past (selected)

1. NIH R21DK070003-01A1 (Gonzalez-Cadavid) NIH NIDDK 10/01/07-09/30/09  
Cell-selective expression of fibrotic gene pathways (no cost extension to 09/30/10)
2. RO1 DK53069-08 (PI: Gonzalez-Cadavid) NIH/NIDDK 05/01/03-04/30/08  
Erectile Dysfunction and Nitric Oxide Synthase in Aging. Renewal to be resubmitted
3. G12RR030262 NIH (PI: Francis/Baker; Core Director: Gonzalez-Cadavid) 09/01/00-08/31/07  
RCMI Infrastructure Development Grant: DNA Repository and Molecular Medicine Core
4. U54 HD41748-01 NIH/NICHHD (PI: Bhasin; PI Pilot grant: Gonzalez-Cadavid) 10/01/03-09/30/07  
Androgen Stimulation of Myogenic Stem Cell Differentiation"
5. Takeda North America, Inc (PI: Gonzalez-Cadavid) 04/01/08-03/31/08  
Antifibrotic and Renoprotective Effects of Pioglitazone on Type 2 Diabetes Related Tubulointerstitial Fibrosis
6. Harbor/UCLA Division of Urodynamics (PI: Gonzalez-Cadavid /Bathia/Ho) 03/01/07-04/01/08  
Reversion of levator ani atrophy by muscle derived stem cells in a rat model of stress urinary incontinence
7. American Diabetes Association (PI: Gonzalez-Cadavid) 08/01/05-07/31/08  
Effect of sildenafil and muscle derived stem cells on cardiac fibrosis after myocardial infarction
8. Lilly ICOS (PI: Gonzalez-Cadavid/Rajfer) 06/01/05-05/31/06  
Effect of tadalafil in preserving smooth muscle function following cavernosal nerve injury

**BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME RAJFER, JACOB	POSITION TITLE Chief of Urology, Harbor-UCLA Medical Center		
eRA COMMONS USER NAME (credential, e.g., agency login) JRAJFER			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
University of Illinois, Chicago, Illinois	B.S.	1968	Biology
Northwestern University, Chicago, Illinois	M.D.	1972	Medicine
L.A. County-USC Med Ctr. Los Angeles, CA	Internship	1973	Medicine
St. Joseph's Hospital, Denver, Colorado	Residency	1974	Surgery
The Johns Hopkins Hospital, Baltimore, MD	Residency	1978	Urology

**A. Personal Statement**

I am Professor in the Department of Urology, UCLA David Geffen School of Medicine, and Chief of Urology at the Harbor-UCLA Medical Center, and as urologist I have a long standing and strong interest in erectile dysfunction. I proposed the idea that nitric oxide was the mediator of penile erection, that led to the first publication in the literature (1990) proving this hypothesis. I have been a coinvestigator in all the translational basic science research on erectile dysfunction/penile fibrosis research, and specifically in the role of the nitric oxide/cGMP pathway, emanating from our laboratory at LABiomed/Harbor-UCLA. I will collaborate with this exciting project, by participating in laboratory discussions and analysis of the results, and will provide the insight on the clinical aspects of the research. This approach should validate the Oct-4 Pr-gfp mouse with streptozotocin-induced diabetes as the first model for direct fluorescence visualization of the endogenous stem cells in the penile corpora cavernosa and the effects of diabetes on their proliferation and differentiation.

**B. Positions and Honors.**

1978-80 Chief of Urology, Veterans Administration Med. Center, Seattle, Washington  
 1978-80 Assistant Professor, Dept. of Urology, University of Washington, Seattle, Washington  
 1980-83 Assistant Professor, Division of Urology, Department of Surgery, University of California, Los Angeles, California  
 1983-89 Associate Professor, Division of Urology, Department of Surgery, University of California, Los Angeles, California  
 1989-07 Professor, Department of Urology, University of California, Los Angeles, California

1977 3rd Prize, Laboratory Research, Garyson Carrol Essay, American Urological Asso., Chicago, IL  
 1978 1st. Prize, Laboratory Research, Garyson Carrol Essay, American Urological Asso., Chicago, IL  
 1983 2nd. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Vancouver, BC  
 1984 1st. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Reno, NV  
 1985 1st. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Anaheim, CA  
 1986 Honorable Mention, Research Prize Section on Urology, Am. Acad. of Pediatrics, Washington, DC  
 1992-07 Best Doctors in America

**C. Selected peer-reviewed publications**

(from 212 peer reviewed publications)

**Most relevant (multipotent/stem cells; diabetes rodent models; penile fibrosis; erectile dysfunction)**

1. Vernet D, Nolzco G, Cantini L, Magee TR, Qian A, **Rajfer J**, Gonzalez-Cadavid NF (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod, 73:1199-1210. PMID: 1609336
2. Kovanecz I, Ferrini MG, Vernet D, Nolzco G, **Rajfer J**, Gonzalez-Cadavid NF (2006). Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. BJU Int, 98:116-124. PMID: 16831155
3. Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, **Rajfer J**, Gonzalez-Cadavid NF (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. J Sex Medic, 4(3):633-43.
4. Kovanecz I, Ferrini MG, Davila HH, **Rajfer J**, Gonzalez-Cadavid NF (2007) Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. BJU Int. 2007 Oct; 100(4):867-74.
5. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, **Rajfer J**, Gonzalez-Cadavid NF (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. Biol Reprod, 76:915-923
6. Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, **Rajfer J**, Gonzalez-Cadavid NF (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res, 2007, 100(4):867-74.
7. Rambhatla A, Kovanecz I, Ferrini M, Gonzalez-Cadavid NF, **Rajfer J** (2008) Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. Int J Impot Res. 20(1):30-4.
8. Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, **Rajfer J**, Gonzalez-Cadavid NF (2008) Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med, 5(7):1607-22
9. Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, **Rajfer J**, Gonzalez-Cadavid NF (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. BJU Int 101(2):203-10.
10. Nolzco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, **Rajfer J**, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int, 101(9):1156-64. PMID: 18294308
11. Kovanecz I, Nolzco G, Ferrini MG, Toblli, JE, Heydarkhan S, Vernet D, **Rajfer J**, Gonzalez-Cadavid NF (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. BJU Int, 2009, 103(10):1396-404. PMID: 19154511
12. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, **Rajfer J**, Gonzalez-Cadavid NF (2009). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Medic, 6(2):415-28
13. Ferrini MG, Moon J, Rivera S, Vernet D, **Rajfer J**, Gonzalez-Cadavid NF (2010) The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. J Sex Med, in press
14. Gonzalez-Cadavid NF, **Rajfer J** (2009) Experimental models for Peyronie's disease. Implications for therapy. J Sex Medic, 6(2):303-13
15. Gonzalez-Cadavid NF, **Rajfer J** (2010) Treatment of Peyronie's disease with PDE5 inhibitors: an antifibrotic strategy. Nat Rev Urol.7(4):215-21. PMID: 20212516

**Other (2005-2010, including erectile dysfunction)**

Davila H, Magee TR, **Rajfer J**, Gonzalez-Cadavid NF (2005). Peyronie's disease is associated with an increase of plasminogen activator inhibitor-1 (PAI-1) at the RNA and protein levels. Urology. 65:645-648

Gore JL, Swerdloff RS, **Rajfer J**. (2005) Androgen deficiency in the etiology and treatment of erectile dysfunction. Urol Clin North Am 32:457-468

Gonzalez-Cadavid NF, **Rajfer J** (2005) The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. Curr Pharm Des 11:4041-4046

Gonzalez-Cadavid NF, **Rajfer J** (2005) Mechanisms of disease: new insights into the cellular and molecular pathology of Peyronie's disease. *Nature Clin Pract Urol*, 2:291-298.

Ferrini MG, Kovanecz I, Nolzco G, **Rajfer J**, Gonzalez-Cadavid NF (2006) Effects of long-term treatment with vardenafil on the development of the fibrotic plaque in a rat model of Peyronie's disease *BJU Int*, 97:625-633

Vernet D, Magee TR, Qian A, **Rajfer J**, Gonzalez-Cadavid NF (2006) Long-term continuous incubation with high doses of tadalafil does not up-regulate the levels of phosphodiesterase 5 (pde5) in cultures of human penile smooth muscle cells. *J Sex Med* 3:84-94; discussion 94-95

Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, **Rajfer J**, Gonzalez-Cadavid NF (2006). Myostatin shRNA gene therapy increases muscle mass. *J Gene Med* 8:1171-81

Ferrini MG, Davila H, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF\* **Rajfer J**\* (\*: equal contributors) (2006) Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. *Urology*, 2006 Aug;68(2):429-35.

Ferrini MF, Davila HH, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF and **Rajfer J**. (2006) Vardenafil prevents the fibrosis and loss of corporal smooth muscle following bilateral cavernosal nerve resection in the rat. *Urology* 68:429-435.

Shabsigh R, **Rajfer J**, Aversa A, Traish AM, Yassin A, Kalinchenko SY and Buvat J. (2006) The evolving role of testosterone in the treatment of erectile dysfunction. *Int J Clin Pract* 60:1087-1092.

**Rajfer J**, Aliotta PJ, Steidle CP, Fitch WP, Zhao Y and Yu A. (2007) Tadalafil dosed once a day in men with erectile dysfunction: a randomized, double-blind, placebo controlled study in the United States. *Intl J Imp Res* 19:95-103.

Gonzalez-Cadavid NF, **Rajfer J** (2007) Experimental models for the study of the cellular and molecular pathophysiology of Peyronie's disease. In: *Current Clinical Urology: Peyronie's Disease, A Guide to Clinical Management*, ed by L.A. Levine, Humana Press, Totowa, NJ, p 19-39

Porst H, **Rajfer J**, Casabé A, Feldman R, Ralph D, Vieiralves LF, Esler A, Wolka AM, Klise SR. (2008) Long-term safety and efficacy of tadalafil 5 mg dosed once daily in men with erectile dysfunction. *J Sex Med* 5(9):2160-9. PMID: 18557812

## C. Active and Completed Funding

R01 DK53069

05/03-04/08

NIH/NIDDK

Erectile Dysfunction and Nitric Oxide Synthase in Aging

To apply novel procedures of gene and stem cell therapy for the treatment of aging related erectile dysfunction based on modulation of the NO/cGMP pathway in the corporal tissue of the rat.

Role: Co-I 3% (non compensated)

N737500/53-5128-4380

01/05-12/07

Los Angeles County/Lance Armstrong Foundation

Los Angeles County Germ Cell Tumor & Tissue Bank Resources at USC

To develop a tissue bank of testis tumors in Los Angeles County for future use by scientific investigators.

Role: PI 2% (non compensated)

R21 DK070003

07/07-06/09

NIH/NIDDK

Cell-Selective Expression of Fibrotic Gene Pathways

To compare the patterns of gene expression related to fibrosis in the smooth muscle and fibroblasts of the corpora cavernosa and tunica albuginea, respectively, of the penis and to determine the relationship between stem cells, smooth muscle cells and fibroblasts in the generation of myofibroblasts in fibrosis.

Role: Co-I 3% (non compensated)

American Diabetes Association

08/05-07/08

Erectile dysfunction and vascular fibrosis in diabetes

To evaluate the role of NO/cGMP in preventing the fibrosis of the corporal tissue and the media of the arterial wall in diabetes mellitus.

Role: Co-I 2% (non compensated)

PR 064756

03/07-02/10

Department of Defense

Pharmacological prevention and reversion of erectile dysfunction after radical prostatectomy by modulation of the NO/cGMP pathways.

To evaluate the role of NO and cGMP in preventing cellular apoptosis and fibrosis in the corporal tissue following cavernosal nerve damage.

Role: Co-I 5% (non-compensated)

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME NOLAZCO, Gaby		POSITION TITLE Research Associate	
eRA COMMONS USER NAME (credential, e.g., agency login)			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
California State University, Fullerton	M.Sc.	2003	Biological Science

**A. Positions and Honors.**

2004-2007 Research Associate, Dept Surgery, Urology Research Laboratory. Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance CA.

2007- on Research Associate, Charles Drew University, School of Medicine, Los Angeles CA.

**B. Publications.**

**1. Nolzco GM**, Ramirez J. (2000) Determination of specific acetylcholine receptor subunit expression in identified neurons found in the marine mollusk, *Aplysia californica*. *Dimensions*, 2: 14-19. (Undergraduate student research publication at CSU Fullerton)

**2. Nolzco GM**. (2003) Identification and developmental expression of alternatively spliced transcripts of the small conductance calcium-activated potassium channel in *Drosophila melanogaster*, Master's Thesis, California State University Fullerton.

**3. Vernet D, Nolzco G**, Cantini L, Magee TR, Qian A, Rajfer J, Gonzalez-Cadavid NF. (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for peyronie disease. *Biol Reprod*, 73(6): 1199-210.

**4. Vernet D, Magee TR, Qian A, Nolzco G**, Rajfer J, Gonzalez-Cadavid NF. (2006) Phosphodiesterase type 5 is not upregulated by Tadalafil in cultures of human penile cells. *J Sex Medicine*, (3(1): 4-95.

**5. Ferrini MG**., Kovanecz I, **Nolzco G**, Rajfer J, Gonzalez-Cadavid NF (2006) Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *Br J Urol*, 97 (3): 625-33.

**6. Kovanecz I, Ferrini MG, Vernet D, Nolzco G**, Rajfer J., Gonzalez-Cadavid NF (2006) Pioglitazone prevents corporal veno-occlusive dysfunction (CVD) in a rat model of type 2 diabetes mellitus. *Br J Urol*, 98(1):6-24.

**7. Ferrini MG, Nolzco G**, Vernet D, Gonzalez-Cadavid NF, Berman J. (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase, in a diabetic rat model: implications for vaginal fibrosis. *J Fertil and Steril*, 86 Suppl 4: 1152-63.



8. Kovanecz I., Ferrini MG, Vernet D, **Nolazco G**, Rajfer J, Gonzalez-Cadavid NF (2007) Ageing-related corpora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. Br J Urol, 100 (4) 867-74.
9. **Nolazco G**, Kovanecz I, Vernet D, Tsao J, Ferrini MG., Magee TR, Rajfer J, Gonzalez-Cadavid NF. (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int (9) 1156-64.
10. Kovanecz I, **Nolazco G**, Ferrini MG, Toblli, J, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. (2009) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction BJU Int 103(10):1396-404. Epub 2009 Jan 19.
11. Tsao J , Vernet D, Gelfand RA, Kovanecz I, **Nolazco G**, Gonzalez-Cadavid NF. (2010) The genetic inactivation of myostatin or dystrophin expression inhibits the myogenic differentiation of a population of stem cells from the mouse skeletal muscle. J Endocrinol. submitted
12. Wang J S-C, **Nolazco G**, Kovanecz I, Vernet D, Kopchok GE, Chow S, Keyhani A, White RA, Gonzalez-Cadavid NF (2010) Effects of muscle derived stem cells and long-term treatment with a PDE5 inhibitor on myocardial infarction in a rat model. Cardiovascular Res, submitted

**C. Active and Completed Funding**  
None

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME KOVANECZ, ISTVAN	POSITION TITLE Assistant Researcher		
eRA COMMONS USER NAME IKOVANECZ0308			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Szeged (former JATE), Szeged, Hungary	M.Sc.	1985	Biochemistry
Budapest University of Technology, Institute of Continuing Engineering Education, Budapest, Hungary	CNRT	1987	Nuclear technology
University of Szeged (former JATE), Szeged, Hungary	Ph.D.	1994	Comparative Physiology
Biological Research Center of The Hungarian Academy of Sciences, Szeged, Hungary		1999-2000	Genomics, IT, Bioinformatics

**A. Personal statement**

The aim of the study is to demonstrate that the diabetic Oct-4 Pr-gfp mouse is an adequate model for the isolation and in vitro characterization of penile endogenous stem cells and their impairment by hyperglycemia, and also an adequate model for studying the modulation of endogenous penile stem cells by exogenous stem cells in the therapy of diabetes-related corporal fibrosis and erectile dysfunction.

I have extensive expertise on physiology, molecular and cellular biology, focused in the last several years studying mechanism of fibrosis (mainly associated with erectile dysfunction) and complications of diabetes in different tissues of the urogenital and cardiovascular system in various rodent models of diabetes type 1 and 2. Part of these studies was stem cell isolation and implantation into various organs for tissue repair. Based on my expertise I am well qualified to participate in the proposed study.

**B. Academic positions and other experience****Positions**

1985 - 1987 Research Fellow, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

1987 - 1991 Research Scientist, Blood Transfusion Center, Szent-Gyorgyi Albert Medical University, Szeged, Hungary

1991 - 1992 Volunteer, Department of Neurology, Mount Sinai Medical Center, CUNY, New York, NY, USA

1993 - 1999 Senior Research Scientist, Head of the Conscious Animal Experimental Laboratory, Department of Pharmacology and Pharmacotherapy, Szent-Gyorgyi Albert Medical University, Szeged, Hungary

1999 - 2001 Biologist Chief Counselor, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

2000 - 2001 Member of the Computer Software Council of the Hungarian Academy of Sciences

2004 - on Research Associate, Urology Research Laboratory, LABioMed Research Institute at Harbor-UCLA, Torrance, CA, USA

2008-on Assistant Researcher, Department of Urology, UCLA

## Other Experience and Professional Memberships

1982–96 John von Neumann Society of Computer Sciences  
1993–01 Hungarian Genetical Society  
1994–99 Hungarian Society of Cardiology  
1994–99 Hungarian Society for Experimental and Clinical Pharmacology  
1994–99 International Society for Heart Research (European Section)  
2000–01 Computer Software Council of the Hungarian National Academy of Sciences  
2006–on National Geographic Society  
2007–on Sexual Medicine Society of North America  
2007–on International Society of Sexual Medicine  
2008–on American Urological Association  
2008–on LABioMed

## C. Selected peer-reviewed publications

### Most relevant (multipotent/stem cells; diabetes rodent models; erectile dysfunction)

1. **Kovanecz I**, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int*. 2006; 98:116-24. PMID: 16831155
2. Ferrini MG, Davila HH, **Kovanecz I**, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. *Urology* 2006; 68:429-35. PMID: 16904479
3. Ferrini MG, **Kovanecz I**, Sanchez S, Vernet D, Davila HH, Rajfer JA, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. *Biol Reprod*. 2007; 76(5):915-23. Epub 2007 Feb 7. PMID: 17287493
4. Magee TR, **Kovanecz I**, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF. Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med* 2007; 4(3):633-43. Epub 2007 Apr 13. PMID: 17433082
5. **Kovanecz I**, Ferrini MG, Vernet D, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Aging-related corpora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. *BJU Int* 2007; 100(4):867-74. PMID: 17662078
6. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. Long term sildenafil treatment ameliorates corpora veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res* 2008 Mar-Apr; 20(2):202-12. Epub 2007 Sep 20. PMID: 17882231
7. Rambhatla A, **Kovanecz I**, Ferrini M, Gonzalez-Cadavid NF, Rajfer J. Rationale For PDE5 Inhibitor Use Post Prostatectomy. *Int J Impot Res* 2008 Jan-Feb; 20(1):30-4. Epub 2007 Aug 2. Review. PMID: 17673932
8. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. *BJU Int* 2008 Jan; 101(2):203-10. Epub 2007 Sep 20. PMID: 17888043
9. Nolzco D, **Kovanecz I**, Vernet D, Ferrini MG, Gelfand B, Tsao J, Mage T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int* 2008 May; 101(9):1156-64. Epub 2008 Feb 21. PMID: 18294308
10. **Kovanecz I**, Nolzco G, Ferrini MG, Toblli J, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. *BJU Int* 2009 May; 103(10):1396-404. Epub 2009 Jan 19. PMID: 19154511
11. Ferrini MG, **Kovanecz I**, Sanchez S, Umeh C, Rajfer J, Gonzalez-Cadavid NF. (2009) Fibrosis and Loss of Smooth Muscle in the Corpora Cavernosa Precede Corporal Veno-Occlusive Dysfunction (CVOD) Induced by Experimental Cavernosal Nerve Damage in the Rat. *J Sex Med*. 2009 Feb; 6(2):415-28. Epub 2008 Dec 2. PMID: 19138364
12. Habte B, **Kovanecz I**, Nolzco G, Wang SC, Gonzalez-Cadavid NF. Effects of long term sustained elevation of cGMP levels on cell death and fibrosis in the heart of a rat model of type 2 diabetes. *Ethnicity & Disease* 2009; 19(2):S369-70
13. Ho MH, Heydarkhan S, Vernet D, **Kovanecz I**, Ferrini MG, Bhatia NN, Gonzalez-Cadavid NF. (2009)

Skeletal Muscle-Derived Stem Cells Seeded On Small Intestinal Submucosal Scaffolds Stimulate Vaginal Repair In The Rat. *Obstet Gynecol*. 2009 Aug; 114(2, Part 1): 300-309. PMID: 19622991

14. Tsao J, Vernet D, Gelfand R, **Kovanecz I**, Nolzco G, Gonzalez-Cadavid NF. The genetic inactivation of myostatin or dystrophin expression inhibits the myogenic differentiation of a population of stem cells from the mouse skeletal muscle. *J Endocrinol*, submitted

15. Wang J S-C, Nolzco G, **Kovanecz I**, Vernet D, Kopchok GE, Chow S, Keyhani A, White RA, Gonzalez-Cadavid NF (2010) Effects of muscle derived stem cells and long-term treatment with a PDE5 inhibitor on myocardial infarction in a rat model. *Cardiovascular Res*, submitted

### Other selected publications

Bodis-Wollner I, Antal A, **Kovanecz I**. Low-dose scopolamine and acetyl-levo-carnitine dissociate primary from cognitive visual processing in the trained monkey. *Invest Ophth Vis Sci* 1993; 34(4): 1174.

Antal A., **Kovanecz I**, Bodis-Wollner I. Visual discrimination and P300 are affected parallel by cholinergic agents in the behaving monkey. *Physiol Behav* 1994; 56(1) 161-66.

Tagliati M., Bodis-Wollner I., **Kovanecz I**, Stanzione P. Spatial frequency tuning in the monkey retina depends on D2 receptor-linked action of dopamine. *Vision Research* 1994; 34(16):22051-57.

**Kovanecz I**, Csajbok E., Petri I.B. In vitro steroid sensitivity in chronic uremic and kidney transplant patients: HLA - associated susceptibility to steroid treatment. *Nephrology Dialysis Transplantation* 1994; 9(10): 1474-76.

**Kovanecz I**, Petri I.B., Kaiser G. HLA associated lymphocyte panel reactive (cytotoxic) antibody production in dialyzed chronic uremic patients. *Acta Microbiologica Hungarica* 1995; 42(1): 81-84.

**Kovanecz I**, Papp JG., Szekeres L. Increased cardiac workload by adrenoreceptor agonists for the estimation of potential antiischemic activity in a conscious rabbit model. *J Pharmacol Toxicol Methods* 1997; 37(3): 149-59.

Szekeres L, **Kovanecz I**, Papp JG. Delayed cardiac adaptation to stress moderates response to beta-adrenoceptor agonists. *J Mol Cell Cardiol* 1997; 29(5): A134.

**Kovanecz I**, Abraham A, Makay G, et al. Delayed cardiac adaptation to ischaemic stress: Limitation of infarct size in a rabbit model of ischaemia-reperfusion by a single dose of iloprost. *J Mol Cell Cardiol* 1997; 29(5): 89.

Takase H, **Kovanecz I**, Mori T. et al. Acute and anti-ischemic actions of pranipidine in three animal models. *Asia Pacific Journal of Pharmacology* 2003; 16(1): 29-37.

Davila HH, Miranda-Sousa AJ, **Kovanecz I**, et al. Effect of bilateral cavernosal nerve resection on the histological alteration in the penile vascular system. *J Urol* 2005; 173(4S): 288.

Ferrini MG, **Kovanecz I**, Nolzco G, Rajfer J, Gonzalez-Cadavid NF. Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int* 2006 Mar; 97(3):625-33. PMID: 16469038

### D. Research Support

#### Funded, ongoing

Institutional Seed Grant 513552

Kovanecz (PI)

08/20/09-08/19/10

LABioMed at Harbor-UCLA Medical Center

Effects of long-term continuous treatment with cGMP/cAMP modulators on diabetes induced erectile dysfunction and penile tissue alterations.

This study is a novel approach to "fine tune" the complex regulation of erection via repairing/preserving tissue function and structure in diabetic rats by simultaneously targeting different signaling pathways with combination of modulators in subclinical doses.

Role: PI

Subaward of Department of Defense PC061300

Gonzalez-Cadavid (PI)

at LABioMed at Harbor-UCLA Medical Center 12914-04 Kovanecz (PI) 03/01/10-02/28/11

Modulation of stem cell differentiation and myostatin as an approach to counteract fibrosis in muscle dystrophy and regeneration after injury.

Role: PI

**BIOGRAPHICAL SKETCH**

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person **DO NOT EXCEED FOUR PAGES**

NAME VERNET, Agueda Dolores		POSITION TITLE Research Associate	
eRA COMMONS USER NAME (credential, e.g., agency login) avernet0507			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Central Univ of Venezuela, Caracas, Venezuela	Biologist	1976	Cellular Biology
Venezuelan Institute Scientific Res, Venezuela	M.Sc.	1983	Immunology
Central Univ of Venezuela, Caracas, Venezuela	Ph.D.	1993	Cellular Biology

**A. Personal Statement**

I am a cell biologist who has been involved for the last 19 years with the study of the pathophysiology of erectile dysfunction and penile fibrosis, particularly in diabetes rodent models, and lately I became involved with stem cell research. Specifically, I was first author on a paper that showed the presence of stem cells in cultures obtained from the human penile tunica albuginea, later collaborated in papers related to the use of muscle derived stem cells (MDSC) for aging-related erectile dysfunction and vaginal repair in rat models, and ongoing I am responsible for the detection of endogenous stem cells (OPSC) in the penile corporal cavernosa of the Oct-4 Pr-gfp mouse. Therefore, this project gives me the exciting possibility of continuing those studies within the UCLA research group and characterize the interaction of both OPSC and MDSC in the diabetic Oct-4 Pr gfp mouse.

**B. Positions**

1976-83 Biologist, Institute Legal Medicine, Caracas, Venezuela  
 1980-81 Visiting Scientist, University of Compiègne, Compiègne, France  
 1983-84 Biologist, Amazonic Center Res Tropical Diseases, Caracas, Venezuelas –  
 Instructor Professor, Dept Biochem, Fac Medicine, Central Univ Venezuela, Caracas, Venezuela.  
 1991 Research Associate, Dept Surgery, Res Education Institute at Harbor-UCLA Medical Center, Torrance, California  
 1992 Assoc Professor, Dept Pathophysiol, Fac.Medicine, Central Univ Venezuela, Caracas, Venezuela.  
 1993-on Research Associate, Dept Surgery, Div Urology, Los Angeles Biomedical Research Institute at Harbor-UCLA Med Center, Torrance, CA  
 2004-2010 Research Associate (part time), DOD Program, Charles Drew University, Los Angeles, CA  
 2010-on Research Associate (part time), Cancer Research Program, Charles Drew University, Los Angeles, CA

**C. Selected Peer-Reviewed Publications**

1. **Vernet D**, Nolzco G, Cantini L, Magee TR, Qian A, Rajfer J, Gonzalez-Cadavid NF (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod, 73:1199-1210. PMID: 16093362

2. Ferrini MG, Nolasco G, **Vernet D**, Gonzalez-Cadavid NF\*, Berman J\* (\*: equal contributors) (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. *Fertil Steril*, 86:1152-1163. PMID: 16978624
3. Kovanecz I, Ferrini MG, **Vernet D**, Nolasco G, Rajfer J, Gonzalez-Cadavid NF (2006). Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int*, 98:116-124. PMID: 16831155
4. Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, **Vernet D**, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Medic*, 4(3):633-43. PMID: 17433082
5. Kovanecz I, Rambhatla A, Ferrini MG, **Vernet D**, Sanchez S, Rajfer J, Gonzalez-Cadavid NF (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res*, 100(4):867-74. PMID: 17882231
6. Kovanecz I, Rambhatla A, Ferrini MG, **Vernet D**, Sanchez S, Rajfer J, Gonzalez-Cadavid NF (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. *BJU Int* 101(2):203-10. PMID: 17888043
7. Nolasco G, Kovanecz I, **Vernet D**, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int*, 101(9):1156-64. PMID: 18294308
8. Kovanecz I, Nolasco G, Ferrini MG, Toblli JE, Heydarkhan S, **Vernet D**, Rajfer J, Gonzalez-Cadavid NF (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. *BJU Int*, 2009, 103(10):1396-404. PMID: 19154511
9. Cantini LP, Ferrini MG, **Vernet D**, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF (2008) Pro-fibrotic role of myostatin in Peyronie's disease. *J Sex Med*, 5(7):1607-22. PMID: 18422491
10. Toblli JE; Ferrini MG; Cao G; **Vernet D**; Angerosa M; Gonzalez-Cadavid NF (2009) Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrol Dial Transpl*, Aug;24(8):2384-91. PMID: 19297362
11. Ferrini MG, Kovanecz I, Sanchez S, **Vernet D**, Umeh C, Rajfer J, Gonzalez-Cadavid NF (2009). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Medic*, 6(2):415-28. PMID: 17287493
12. Ho MH, Heydarkhan S, **Vernet D**, Kovanecz I, Ferrini MG, Bhatia NN, Gonzalez-Cadavid NF (2009) Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds. *Obst Gynecol*, Aug;114(2, Part 1):300-309 PMID: 19622991
13. Ferrini MG, Moon J, Rivera S, **Vernet D**, Rajfer J, Gonzalez-Cadavid NF (2010) The genetic inactivation of inducible nitric oxide synthase (iNOS) intensifies fibrosis and oxidative stress in the penile corpora cavernosa in type 1 diabetes. *J Sex Med*, in press
14. Tsao J, **Vernet D**, Gelfand R, Kovanecz I, Nolasco G, Gonzalez-Cadavid NF. (2010) The genetic inactivation of myostatin or dystrophin expression inhibits the myogenic differentiation of a population of stem cells from the mouse skeletal muscle. *J Endocrinol*, submitted
15. Wang J S-C, Nolasco G, Kovanecz I, **Vernet D**, Kopchok GE, Chow S, Keyhani A, White RA, Gonzalez-Cadavid NF (2010) Effects of muscle derived stem cells and long-term treatment with a PDE5 inhibitor on myocardial infarction in a rat model. *Cardiovascular Res*, submitted

## **Other Publications**

San Blas G, **D Vernet** (1975) Induction of the synthesis of the cell wall  $\alpha$ -1, 3-glucan in the yeast-like form of Paracoccidioides brasiliensis strain IVIC Pb9 by fetal calf serum. Infect & Immun 15:897-902.

- Gonzalez-Cadavid NF, **D Vernet**, A Fuentes, JA Rodriguez, RS Swerloff, J Rajfer (1993) Up-regulation of levels of androgen receptor and its mRNA by androgens in smooth-muscle cells from rat penis. Molec Cell Endocrinol 90:219-229
- Garban H, **D Vernet**, A Freedman, J Rajfer, NF Gonzalez-Cadavid (1995) Effect of aging on nitric oxide-mediated penile erection in rats. Am J Physiol 268: (Heart Circ Physiol) H467-H475
- Hung A, **D Vernet**, Y Xie, T Rajavashisth, JA Rodriguez, J Rajfer, NF Gonzalez-Cadavid (1995) Expression of the inducible nitric oxide synthase in smooth muscle cells from the rat penile corpora cavernosa. J Androl 16:469-481
- Vernet D**, L Cai, H Garban, ML Babbitt, F Murray, J Rajfer, NF Gonzalez-Cadavid (1995) Reduction of penile nitric oxide synthase in diabetic BB/WOR<sup>dp</sup> (Type I) rats with erectile dysfunction. Endocrinology 136:5709-5717.
- Garban H, D Marquez, T Magee, J Moody, T Rajavashisht, JA Rodriguez, A Hung, **D Vernet**, J Rajfer, NF Gonzalez-Cadavid (1997) Cloning of rat and human inducible nitric oxide synthase. Application for gene therapy of erectile dysfunction. Biol Reproduct 56, 954-963.
- Moody J, **Vernet D**, S Laidlaw, J Rajfer, NF Gonzalez-Cadavid (1997) Effects of long-term administration of L-arginine on the rat erectile response. J Urol 158, 942-947
- Vernet D**, Bonavera JJ, Swerloff R, Gonzalez-Cadavid NF, Wang C. (1998) Spontaneous expression of inducible nitric oxide synthase (iNOS) in the hypothalamus and other brain regions of aging rats. Endocrinology, 139(7): 3254-3261
- González-Cadavid NF, Burnett AL, Magee T, Zeller CB, **Vernet D**, Gitter J, Smith N, Rajfer J (2000) Expression of penile neuronal nitric oxide synthase variants in the rat and mouse penile nerves. Biol Reprod, 63:704-714
- González-Cadavid NF, Ryndin I, **Vernet D**, Magee TR, Rajfer J (2000) Presence of NMDA receptor subunits in the male lower urogenital tract. J Androl 21:566-578
- Ferrini M, Magee TR, **Vernet D**, Rajfer J, Gonzalez-Cadavid NF (2001) Aging-related expression of inducible nitric oxide synthase (iNOS) and markers of tissue damage in the rat penis. Biol Reprod, 64:974-982
- Wang C, Sinha Hikim A, Ferrini M, Bonavera JJ, **Vernet D**, Leung A, Lue-YH, Gonzalez-Cadavid NF, Swerloff RS (2002) Male reproductive aging: using the Brown Norway rat as a model for man. In: "Endocrine facets of aging in the human and experimental animal" Novartis Found Symp 242:82-95
- Ferrini MG, **Vernet D**, Magee TR, Shahed A, Quian A, Rajfer J, Gonzalez-Cadavid NF (2002) Antifibrotic role of inducible nitric oxide synthase (iNOS). Nitric Oxide 6:1-12
- Gonzalez-Cadavid NF, Magee TR, Ferrini M, Qian A, **Vernet D**, Rajfer J (2002) Gene expression in Peyronie's disease. Int J Impot Res, 14:361-374.
- Magee TR, Ferrini M, Garban H, **Vernet D**, Mitani K, Rajfer J, Gonzalez-Cadavid NF (2002) Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase cDNA. Biol Reprod, 67:1033-1041.
- Vernet D**, Ferrini MG, Valente E, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF (2002) Effect of nitric oxide on fibroblast differentiation into myofibroblasts in cell cultures from the Peyronie's fibrotic plaque and in its rat model *in vivo*. Nitric Oxide 7:262-276.
- Ferrini MG, Magee TR, **Vernet D**, Rajfer J, González-Cadavid NF (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic and spinal cord regions involved in the control of penile erection. J Compar Neurol 458:46-61
- Magee TR, Ferrini MG, Davila HH, Zeller CB, **Vernet D**, Sun J, Lalani R, Burnett AL, Rajfer J, González-Cadavid NF. (2003) Protein inhibitor of nitric oxide synthase (NOS) and the N-methyl-D-aspartate receptor are expressed in the rat and mouse penile nerves and colocalize with penile neuronal NOS. Biol Reprod 68:478-488.

- Valente EG, **Vernet D**, Ferrini MG, Qian A, Rajfer J, González-Cadavid NF (2003) L-arginine and (PDE) inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. Nitric Oxide, 9:229-244
- Davila HH, Magee TR, **Vernet D**, Rajfer J, González-Cadavid NF. (2004) Gene transfer of inducible nitric oxide synthase cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease. Biol Reprod 71(5):90
- Sinha Hikim AP, Vera Y, **Vernet D**, Castanares M, Diaz-Romero M, Ferrini M, Swerdloff RS, González-Cadavid NF, Wang C. (2005) Involvement of nitric oxide-mediated intrinsic pathway signaling in age-related increase in germ cell apoptosis in male brown-norway rats. J Gerontol A Biol Sci Med Sci. 60(6):702-8.
- Vernet D**, Magee TR, Qian A, Nolasco G, Rajfer J, González-Cadavid NF. (2006) Phosphodiesterase type 5 is not upregulated by tadalafil in cultures of human penile cells. J Sexual Medicine, 3:84-95.
- Magee TR, Artaza JN, Ferrini MG, **Vernet D**, Zuniga FI, Cantini L, Reisz-Porszasz, Rajfer J, González-Cadavid, NF. (2006) Myostatin short interfering hairpin RNA gene transfer increases muscle mass. J Gene Med, 8(9):1171-81.
- Ferrini MG, Kovanecz I, Sanchez S, **Vernet D**, Davila HH, Rajfer J, Gonzalez-Cadavid NF. (2007) Long-Term Continuous Treatment with Sildenafil Ameliorates Aging-Related Erectile Dysfunction and the Underlying Corporal Fibrosis in the Rat. Biol Reprod 76:915-23; [Epub ahead of print]

**D. Active and Completed funding**

None